THE TRAINING OF POSTGRADUATE STUDENTS

A JOINT committee appointed by the Institute of Physics and the Physical Society has prepared an admirable report on the postgraduate training of physicists in British universities. Besides university professors of physics its members included industrialists of the standing of Dr. R. Beeching, who is a director of Imperial Chemical Industries, Ltd., and some twenty other physicists attended one or more of its meetings to give their views. The report* does not claim to be more than a first step in investigating a very big problem, and is, in fact, concerned mainly with problems arising in training those who are working for the Ph.D. degree, including the suitability of that degree as a preparation for fundamental and applied research. It is packed with common sense and, taken with the lecture on "The Education of Chemists" which Prof. T. L. Cottrell delivered at Edinburgh last November, with which the report may appropriately be read, it affords clear evidence of fresh thought about the nature and content of university courses, and indeed of professional courses both inside and outside the universities. In fact, issues are raised if not fully discussed which should certainly be considered by the Royal Institute of Chemistry in determining points of policy arising out of the tentative proposals for a new grade of membership and category of affiliation.

In British universities postgraduate training in science has been essentially preparation for the Ph.D. degree: the bachelor's degree with honours has been, and is still, the qualification which often represents the completion of a future scientist's formal university education, and at this stage many graduates, including some of the ablest, enter government and industrial laboratories for careers which may include original and fundamental research. The view was put to the Committee that in any foreseeable circumstances the recruitment of adequate numbers of Ph.D. students is essential for the vitality of university departments which are actively concerned with the advancement of knowledge. Without questioning the view that the prosecution of original research is a major university responsibility, and recognizing the existence since the Second World War of a large and only partly satisfied demand from industry and from government departments for graduates in physics for research and development, the Committee sees postgraduate training as an aspect of the wider issue of the most effective use of scientific ability of a high level. Its scrutiny is penetrating and some of its comments are strikingly similar to those of Prof. Cottrell on the training of chemists.

The report points out that the training of the research worker in British universities rests upon four assumptions. First, it presupposes enlightened supervision in an atmosphere of maturity. Secondly, it assumes that all the research students have the innate qualities that will allow them to profit by this form of training. Implied in this assumption is successful selection, and that those who gain a research degree will be characterized by manifest research ability and maturity. Thirdly, it assumes that physicists of the highest potential ability will benefit from a broadly similar method of research training whatever their ultimate work may be, though it is recognized that in the past theoretical physicists have tended to come from those trained as mathematicians. Fourthly is the assumption that the present level of the first degree is the appropriate stage at which the basic and common education of a scientist may cease, and that additional knowledge required to be gained beyond this stage is knowledge of a specialized nature determined by the field of research being undertaken.

It is against this background that the Committee examined criticisms made of present practice in Britain, some of which imply a complete denial of one or more of the assumptions. From within the universities the adequacy of the three-year undergraduate course has been questioned and a four-year course has been advocated purely on the ground of the growth of physics itself in recent years. While the report makes no comment on an important point made by Prof. Cottrell that scientific advances, by including more facts in the scope of a wider generalization, may reduce rather than increase the amount of information to be memorized, the Committee clearly recognized serious practical difficulties in increasing the length of undergraduate courses; and the practical suggestion made by the Committee is rather the desirability of a further one-year university course for selected students.

It is the industrial criticism that is strikingly parallel with Prof. Cottrell's comments on the training of chemists. The chief defects in the quality of Ph.D. graduates recruited are lack of scientific originality, lack of good theoretical knowledge of physics and lack of flexibility and adaptability. The first is primarily a criticism of the selection of research students, or of the unwillingness of universities to refuse to award the degree to those once accepted; but it could also be a criticism of poor supervision and of bad selection of problems tackled, which might stifle scientific originality. It is the most serious of the criticisms that can be made against the universities in this respect.

The second criticism is widespread, but is made on two different grounds. First, it is suggested that the Ph.D. graduates sometimes lack adequate theoretical knowledge of even the fields in which they have undertaken their research, though from Prof. Cottrell's

* The Postgraduate Training of Physicists in British Universities: The Report of a Joint Committee appointed by the Institute of Physics and the Physical Society. Pp. 19. (London: Institute of Physics, 1960.) 2s.

criticism of the graduate in chemistry it could be due to failure to apply theory and instead to resort to the purest empiricism. With this could also go the absence of a sufficiently critical and inquiring attitude to a research problem. Secondly, some employers seem to expect the holder of a Ph.D. degree to possess a deeper theoretical knowledge of physics over quite a wide front than could be expected in a graduate with a first degree. That criticism, however, could scarcely be advanced if the graduate had, as Prof. Cottrell insists, been adequately trained and encouraged to read and think for himself and to make adequate use of the literature.

The third criticism is also widely held and certainly strongly held, and though it is the most surprising of the criticisms noted in this report, it finds some support in Prof. Cottrell's observations on chemists. All these criticisms, however, are essentially criticisms of detail and do not necessarily imply t .t university postgraduate training leading to a Ph.D. degree is not fundamentally a highly desirable method of preparation for entry to industry and government service. Much more fundamental criticism is implied in the view held by some important and distinguished industrialists that the necessary preparation for, or apprenticeship to, research is more profitably undertaken in a large industrial laboratory than in the atmosphere of a university research school. Industry has only a limited need for people who will work on difficult problems of pure physics; but it has great need for men of similar intellectual ability and basic training who can apply their abilities and knowledge to the varied problems of industry. These men need to be able to cross with ease the boundaries that conventionally delineate the various sciences taught and organized in universities, and the report notes that the view is widely held that postgraduate training of the Ph.D. pattern may make a man less able or willing to do this. The Committee states that it was put with force that only a small minority, probably fewer than 10 per cent, of those recruited into industry will be engaged on research for the whole of their careers of forty years or so. Evidence was presented that the possession of a Ph.D. degree had no significant bearing upon a future career, as measured, in any event, in financial terms.

It is at this point that the relevance of Prof. Cottrell's comments on the training of chemists becomes most apparent. The demand from industry for men who have had a Ph.D. training is real, but is smaller than the output of such graduates; at present, industry is compelled to take Ph.D.s whom it would have preferred to take at an earlier stage; and it was equally clear to the Committee that the number of Ph.D.s who would be sought by industry might be increased if the average quality, measured by originality, knowledge and flexibility, were raised.

The Committee states its reasoned conclusions in ten paragraphs. First, for its purpose, which is fundamentally the introduction of an undergraduate to the procedure of fundamental research, working for the Ph.D. is an admirable training; but if it is to retain its value as a hallmark of capacity for original research, the universities must be jealous of the high standard of those on whom it is conferred. Next, the research student plays an important part in maintaining the vitality of a university physics department: without a sufficient number of such students the Committee believes it would not be possible to maintain an appropriate output of fundamental research, or to retain or recruit academic staff of suitable character. It points out, however, that the universities must ensure—which is not always the case at present—that research students are not employed partly as high-grade technicians; but it passes off a problem which is very real in some university departments, involving questions of status and prejudice, and it may be wondered whether the implications of the proposals of the Royal Institute of Chemistry to form a Registered Chemical Assistants Group, affiliated to the Institute, have been thought out sufficiently.

It may be recalled that Prof. Cottrell roundly challenged the value of much of the manipulative work customary in the training of the chemist, and his analysis is highly suggestive in respect of the physicist as well as of the physical chemist. This is not to deny the importance of such work, or to suggest that the career of laboratory technician should not be satisfying and highly regarded in its own right: not nearly enough is, in fact, done to encourage young people with manual dexterity and skill to take up such a career. What is wrong is to suggest that the career of laboratory technician is necessarily a stepping-stone to that of scientist or technologist, or that the technician's skill is necessarily linked with high intellectual ability. There may be the odd technician with such gifts which will carry him naturally to a seat on the main board of such a company as Imperial Chemical Industries, Ltd.; but he is the exception, and the technician is best served by honouring his or her special skill in its own right, rather than by encouraging ambitions which will generally be doomed to disappointment.

The appropriate and more extensive use of technicians is a problem which deserves much more attention than it has yet received and itself bears closely alike on the most efficient use of highly trained man-power and on the research output of a university department. Further, it is possible that some way may be found here of diminishing the pressure on the academic staff and thus helping to meet other criticisms of postgraduate training. While the Committee believes that the standard of the Ph.D. degree in physics is at least as high as in most other subjects, it accepts the view that the degree is sometimes awarded to candidates of inadequate calibre and concludes that the universities should be more critical in their selection of research students, and readier to refuse the award, even after the completion of the prescribed period of study and research. There is a strongly held opinion among employers that the standard of the Ph.D. has deteriorated significantly in the past twenty years.

Fourthly, the Committee is satisfied that industry needs physics graduates of wide knowledge, adapt-

ability and first-class intellectual capacity, but that it would prefer that only a small proportion of its recruits had had research training for a Ph.D. degree in a university. Comparatively few jobs in industry will involve research in pure physics, and graduates who contemplate staying at the university for a Ph.D. should appreciate that the degree will not usually improve their prospects of advancement in industry. Some university teachers attribute industry's lack of enthusiasm for the Ph.D. degree to the fact that, in physics, fundamental research is very largely carried out in universities, and the Committee concludes that it would help to resolve the apparently conflicting demands of industry and the universities if more members of university staffs were aware of the problems of industry and the requirements of industrial research, and more industrialists appreciated the crucial place of research and the research student in the modern university.

Next, the Committee concludes that an extension of knowledge, beyond that appropriate to the present first-degree courses, should form part of university training at postgraduate-level, not narrowly centred on an immediate field of research, but such as to strengthen substantially the graduate's background knowledge of that part of physics in which his research is to be done. It is suggested that for most research students this teaching should be concentrated in the first postgraduate year. Many graduates who enter industry without taking a Ph.D. degree would benefit greatly from similar courses, and the Committee suggests the organization of courses so that they could be available to both groups of graduates; this would meet a real industrial need, and it might ease the problem of organization within the universities, and also assist in the assessment of students capable of staying on for research.

Finally, the Committee recognizes that the development of postgraduate courses would aggravate the serious problems of staffing which already face the universities and also that, since most university students are dependent on maintenance grants, no improvement of training on the lines suggested is feasible without provision of the necessary financial support by the grant-awarding bodies, particularly the Department of Scientific and Industrial Research.

As already noted, the Committee limited itself almost exclusively to problems arising in the training of those working for the Ph.D. degree, and it frankly recognizes that there are equally important problems involved in the further training of those who take up professional work in physics immediately after taking a first degree. Investigation of those problems involves consideration of the nature and extent of industry's need of scientific man-power, and it is to be hoped that such an investigation will be undertaken by a committee at least as representative of both the academic and the industrial aspects as that which, under Prof. N. Kemmer's chairmanship, has produced the present report, and before plans for further expansion of university and of technological education are too firmly set. Meanwhile, Prof. Kemmer and his colleagues are to be congratulated on a report which should be of considerable interest not simply to physicists but also to chemists, and to other scientists and their professional organizations, as well as to the universities.

NUCLEAR THEORY AND ITS UTILIZATION

Lectures on Nuclear Theory

By L. D. Landau and Ya. Smorodinsky. Translated from the Russian. Revised edition. Pp. vii+108. (New York: Plenum Press, Inc.; London: Chapman and Hall, Ltd., 1959.) 5.25 dollars; 45*s*. net.

Introductory Nuclear Theory

By Dr. L. R. B. Elton. Pp. xi+286. (London: Sir Isaac Pitman and Sons, Ltd., 1959.) 40*s*. net.

Soviet Reviews of Nuclear Science on the 40th Anniversary of the October Revolution

(Being Vol. 3, No. 11 of *Atomnaya Energiya*, November, 1957.) (International Series of Monographs on Nuclear Energy. Division 2: Nuclear Physics, Vol. 3.) Pp. v+110. (London and New York: Pergamon Press, 1959.) 30*s*. net.

Fundamental Aspects of Reactor Shielding

By Dr. Herbert Goldstein. Pp. xvi+416. (London and New York: Pergamon Press, 1959.) 66*s*. 6*d*. net.

Fundamentals of Nuclear Energy and Power Reactors

By Henry Jacobowitz. Pp. 118. (New York: John F. Rider Publisher, Inc.; London: Chapman and Hall, Ltd., 1959.) 2.95 dollars; 24*s*.

An Introduction to Thermonuclear Research

A Series of Lectures given in 1955. By Albert Simon. (International Series of Monographs on Nuclear Energy. Division 14: Plasma Physics and Thermonuclear Research, Vol. 1.) Pp. ix+182. (London and New York: Pergamon Press, 1959.) 35*s*. net.

THE six books listed represent a mixed bag. "Lectures on Nuclear Theory" by Landau has appeared before and is now available in book form, surprisingly without the name of the translator. The lectures are extremely clearly presented: they were, in fact, originally delivered to experimental physicists and the authors have been at great pains to describe most carefully the physical pictures they analyse mathematically, and to state the limit to which modern experimental results allow theory to go. What we have learned about forces between nucleons from scattering experiments occupies half of the book. Then follows a detailed statement of structure of light and heavy nuclei and a review of neutron and meson interaction with nuclei. A delightful book, not only for students but also for all physicists who enjoy clear exposition. Dr. Elton's "Introductory Nuclear Theory" covers the same ground in much greater detail and with more extensive mathematical proof. It is a text-book for the theoretical physicist of final honours and postgraduate standard and as such is an extremely thorough presentation based, as Dr. Elton describes in his preface, on the phenomenological approach to the nuclear data emanating from the large machines and cosmic ray plates.

The "Soviet Reviews of Nuclear Science" is rather old: the papers were prepared to mark the 40th anniversary of the October Revolution. Pontecorvo

writes "In the glorious fortieth year of the October . . ."! The four papers on nuclear power in the U.S.S.R. are completely superseded by the Geneva 1958 papers and were not worth reprinting at this date: this might also be true of the two survey articles on radioisotopes and radiochemistry. It seems rather pointless to translate review articles; reviews on most subjects are prepared in many countries and the review here translated on interaction of slow neutrons with nuclei is based on world literature, and again all the material can be found in the Geneva papers. There remains one long article on research with the 6-metre synchro-cyclotron, a review article of the Soviet paper with no reference to foreign work. However, this is a valuable summary of experimental results and is well illustrated.

Two books on reactors stand at the top and bottom of their class. Goldstein's authoritative work on "Fundamental Aspects of Reactor Shielding" comes from the Division of Reactor Development of the Atomic Energy Commission, and clearly illustrates the great part the research physicist has to play in nuclear engineering. Reactor shielding is an expensive fraction of nuclear power costs, and "the proper design for a given reactor shield should start from first principles". This book deals very thoroughly with first principles, neutron and gamma-ray attenuation in isotopic and discontinuous media, and compares theory with the experimental results achieved over the past few years with the shielding facilities built on to the Oak Ridge reactor, the graphite and the original water-moderated 'pool'-type reactor. These facilities and the instrumentation for shield testing are well described; the latter illustrates how alert the experimenter must be in this subject if measurements in high and low fluxes are to be reasonably accurate. The book opens with a careful discussion of the biological effects of radiation. The reader notes the frequent references to results reported in Oak Ridge and other laboratory reports, apparently not submitted to any of the learned societies or established journals. Many were originally classified and appear now with references declassified or even still classified: the abstracting journals will be presented with a serious problem if this practice continues. The book will be essential to all engaged on shielding studies, and the references to original work are comprehensive.

One wonders for whom "Fundamentals of Nuclear Energy and Power Reactors" has been written, for it is neither comprehensive nor superficially accurate; but it sets out to attract the general interested public and the members of the atomic industry. For the latter, it does not even mention the important developments abroad, though the book does not state that it is merely reviewing the American scene. It dismisses the graphite-moderated natural uranium reactors as obsolescent, in spite of more than 4,000 MW. of such reactors under construction, and makes no reference to the Russian reactor incorporating the superheating of steam by nuclear energy. The book is well illustrated, but alas contains much slang—do we get "mileage" out of fuel? I would commend the author to Dr. Goldstein's impeccable style.

The appearance of "An Introduction to Thermonuclear Research" in 1959 in its present format is a little disappointing; the Geneva 1958 papers disclosed so much more information than can be found in this book. The preface states that the book is based on lectures of 1955 to which some 1956 information has been added: the basic science in the book is worthy of inclusion although it can all be found elsewhere, but the long catalogue of the American devices in which plasma studies have been made will be nearly meaningless to scientists not working in the field and is outdated information for those who are. The book makes no reference to work in other countries as it closes in 1956: the reviews in the Geneva 1958 papers (Volume 31) will repay study better than this little volume. T. E. Allibone

CONSTANTS GALORE

The Physico-chemical Constants of Binary Systems in Concentrated Solutions

By Prof. Jean Timmermans. Vol. 1: Two Organic Compounds (without Hydroxyl Derivatives). Pp. xiii+1259. Vol. 2: Two Organic Compounds (at least one a Hydroxyl Derivative). Pp. xi+1272. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1959.) 218*s*. a volume.

THESE two books are part of a series of four volumes prepared as a result of a systematic literature survey carried out between 1910 and 1957. Unfortunately no list of the journals abstracted is given. The National Science Foundation of Washington, D.C., gave a substantial grant to enable Dr. Lewin to have the records typewritten so that the publishers could produce the books by offset printing. The pages contain an immense amount of information, but they are disfigured by lack of uniformity; for example, the negative sign sometimes appears before every entry in a table but in other places it appears before the first entry only although it is applicable to all the entries. The arrangement of the mixtures follows a scheme devised by the author, but as the index and bibliography are reserved for volume 4 search for information can only be made by turning pages. Some assistance is afforded by the pair of names boldly printed at the head of each page and these usually refer to a mixture occurring somewhere upon that page, but unfortunately in some instances (for example, Volume 1, pp. 765 and 1191) the names do not coincide with the mixtures listed.

Once a mixture is located the existing data are found arranged in the following order: heterogeneous equilibria (critical constants, saturated vapour pressure curve, boiling curve, azeotropes, composition of liquid and vapour in co-existing phases, densities and rectilinear diameters, composition of the two liquid phases, critical solution point, freezing and melting curve, eutectic and transition points, equilibria of condensed phases under high pressure); properties of phases, first for the gas, then for the liquid and finally for mixed crystals (density, coefficient of expansion, compressibility, viscosity, surface tension, refractive index, optical dispersion, dielectric constant, electrical conductivity, optical rotating power, magnetic rotation, magnetic susceptibility); thermal constants (specific heat, heat of solution or mixing, heat of vaporization and fusion, thermal conductivity). All numerical data have been taken directly from the original papers and values read from graphs are indicated. A list of symbols and abbreviations is presented at the front and back of each volume, but the symbols do not conform to modern usage. In particular, a reader should note that the old Q convention for heat quantities is adopted.

The words "Systems in Concentrated Solutions" are unusual as applied to binary mixtures because a solution that is concentrated with respect to one component is dilute with respect to the other. The author says, however, that concentrations between 10 and 90 weight per cent are tabulated, although the tables do in fact contain some figures outside this range. The percentages recorded appear to refer to the concentration of the second named component, although this is not stated in the list of symbols or in the instructions to users.

The selection of values is not critical and many of them were originally published long before 1910. Thus, some systems are covered at great length and many discordant values for certain constants are recorded. Timmermans discusses in the preface to Vol. 2 the reasons for these divergent values and suggests that they arise in part because insufficient care has been taken by some authors in the purification of specimens. If the purity of the samples is employed as a criterion of worth, much of the recorded data has little value.

These volumes are expensive, but they provide such a useful guide to published data, particularly for a worker conversant with the substances under investigation, that many libraries will wish to acquire these books. E. F. G. HERINGTON

THE BRAIN IN RESPONSE

Person Perception and Interpersonal Behavior

Edited by Renato Tagiuri and Luigi Petrullo. Pp. xx+390. (Stanford, Calif.: Stanford University Press; London: Oxford University Press, 1958.) 60*s*. net.

The Problems of Perception

By R. J. Hirst. (The Muirhead Library of Philosophy.) Pp. 330. (London: George Allen and Unwin, Ltd.; New York: The Macmillan Company, 1959.) 30*s*. net.

IN recent years, an increasing degree of attention has been devoted to the study of what is called 'person perception' or 'social perception'. The book "Person Perception and Interpersonal Behavior" consists of a symposium of 23 papers on these subjects. As is usual with such symposia, the relationship between the topics discussed in the various papers is often not clear. The value of the book, especially to the general reader, would have been greatly increased by the addition of some attempt to sum up and derive general conclusions from the papers as a whole. The reader will find all the more difficulty in doing this for himself because the terms 'person perception' and 'social perception' are used by different authors to include several different processes the inter-relationship of which has never been worked out. It is generally accepted that we not only perceive, more or less objectively, the physical characteristics and actions of other people; we also infer from these perceptions personal characteristics such as feelings of liking and disliking, motives and intentions, social status and background, etc. The original perceptions, and still more the inferences derived from them, are enormously affected by our own feelings, motives and social relationships to other people, and by the way in which we react to them. Sometimes the effects are relatively consistent, but often they vary according to the particular situation. Included in this book are studies by Ittelson and Slack and by Secord of the factors which affect the perception of faces and other physical characteristics. But there are many more studies of the inferences and judgments made about the personality characteristics of others, and how these vary with the personality and likes and dislikes of the judge, and with the social situation. There is an interesting paper by Jones and Thibaut on how people interact in a variety of social situations; and several papers discussing the reasons for one person being attracted by another —whether because the second fulfils the needs of the first, or because he appears to possess the same personality characteristics or interests. A final paper by Cronbach demonstrates the present limitations on the accurate analysis of these judgments, feelings and interactions, and the factors which determine them.

Little or no attempt appears to have been made to discover what is actually perceived, or what are the significant aspects of behaviour and expression which we observe in others; nor how these perceptions lead to the inferences and judgments described above. Granted this is a task of great difficulty and complexity, but it is hard to see how any real understanding of 'person perception' can be gained until some systematic analysis has been made of the psychological processes involved and their interrelationship. Thus, although the psychologist with some knowledge of these topics can find many points of interest in the papers included in this book, the general reader will be liable to become hopelessly confused by the mass of apparently unrelated hypotheses which are frequently expressed in bewilderingly esoteric language.

"The Problems of Perception" provides a philosophical study of the nature of perception in general. It presents a critical examination of the various philosophical theories as to the nature of perception, together with the author's own theory, which is more in accordance with present-day knowledge of the physiology and psychology of perception. The book should be of real interest and value not only to philosophers but also to psychologists and to the general reader. It is clearly written, and necessitates little previous knowledge of philosophy or of technical language.

The author demonstrates convincingly that earlier theories have never provided a satisfactory explanation for the high degree of veridicality of our perceptions on one hand, in spite of the restricted nature of the physiological information conveyed by the sensory nerves to the brain; and on the other hand, the modifications, errors and individual differences in perception which undoubtedly occur, and the apparently perceptual nature of hallucinations which arise in default of external stimulation. His own theory appears to provide a much better explanation. It makes due allowance both for the convincing objectivity and external reference in our perceptions of the surrounding world, and also for the undoubted influence on them of subjective factors; though whether in fact perception of external objects as such is inherent and arises even in early infancy is not perhaps as certain as he would have it to be.

There is one matter which his theory, depending as it does on the concept of 'perceptual consciousness', does not appear to cover adequately—namely, the unconscious perceptions which seem so frequently to guide our habitual and automatic actions. Psycho-

logical study of this type of perception, and of perception at the margin of consciousness, is only just beginning. But when rather more is known of these perceptions, philosophical theory may require further extension to cover them. M. D. Vernon

STRUCTURE AND GROWTH OF PLANT CELL-WALLS

The Plant Cell-Wall

By Prof. Pieter A. Roelofsen. (Encyclopedia of Plant Anatomy, Band 3, Teil 4.) Pp. vii+335+68 plates. (Berlin-Nikolassee: Gebrüder Borntraeger, 1959.) n.p.

In this age, when man-made fibres are so very much in the news, it is a salutory lesson to recall that naturally occurring fibres were in existence upon the Earth long before man, and that they continue to be manufactured by living organisms with a perfection and in a complexity which man still cannot match. Such a lesson is given by Roelofsen's book for fibres made by plants.

The structure of the plant cell-wall has been under constant review ever since its observation by Robert Hooke in 1664, first by light microscopy then, largely after the influence of Carl v. Naegeli, by polarization microscopy, then by X-ray diffraction analysis and now by all these methods supplemented by electron microscopy. Up to this newest development, the study was in the hands of the relatively few botanists with the requisite knowledge of physical principles and training in physical techniques. The electron microscope has, however, thrown the field wide open, and this, coupled with the growing realization of the importance, both academic and technological, of the cell-wall, has led, during the past ten years, to a vast increase in the number of laboratories actively engaged in research and therefore in the number, range and variety of published papers. Prof. Roelofsen himself returned to this study some ten years ago after an absence of twenty years and has during this period of rapid expansion already established himself as an authority in this difficult field.

This book, in my opinion in many ways the best which has yet appeared on this subject, can only enhance Prof. Roelofsen's reputation. The deep appreciation of the powers of electron microscopy which all workers feel make it dangerously easy to fall into the trap of ignoring older work by older methods. This trap Roelofsen conscientiously avoids. It is, indeed, one of the most admirable features of his book that each topic he takes up is dealt with historically, in the sense that what may be called the classical key investigations are reviewed in detail and the later work is always placed in its proper context against this general background. To my certain knowledge very few lines of investigation are omitted and very few papers of lasting importance fail to receive due appreciation. Perhaps the biosynthesis, particularly of cellulose, does not have the attention it appears to merit in view of its importance in questions of wall growth, but this may be due to shortage of space.

The book is divided broadly into two sections. The first, in eight subsections covering 100 pages, deals with the nature, occurrence, chemical and physical properties and biosynthesis of the various components of plant cell-walls. This includes treatment not only of the 'standard' components—celluloses, hemicelluloses, polysaccharides, lignin, and chitin but also of low molecular weight polyoses, cutin, suberin and rarer compounds such as phytomelane. There is in these pages much useful information which, so far as I am aware, has not hitherto been gathered together in one volume.

The second, larger, section, in four subsections covering more than 200 pages, deals with the physical methods of investigation and a wide review, surprisingly detailed and comprehensive in view of the comparatively small space available, of wall structure in higher plants, in algae and in fungi. The treatment of the physical methods is, wisely, not detailed; even making allowances for difficulties in translation the text in various places makes it clear that Prof. Roelofsen is himself not a physicist and he refers his readers to other texts. There is, however, sufficient here to enable the intelligent reader to make a critical appraisal of the subsequent statements. As to these, it is not an exaggeration to say that the facts available up to the time of going to press are all here, clearly stated and documented by reference to the appropriate literature. Equally, both the older and the current theories concerning mechanisms of orientation and of surface growth of the primary walls in higher plants are dealt with, though here the treatment is not, perhaps, so comprehensive. Prof. Roelofsen is himself the author of a theory of growth which is very much to the fore at this moment and it is natural, and welcome, that he should pay to this a good deal of attention in his book. I find much in the hypotheses formulated in this part of the book to which I could not lend support—the author, for example, seems to repeat an older error in confusing *stress* with *strain* and this leads to a peculiar paragraph on p. 175 (the first paragraph in 228231)—but this again is natural and no criticism; the exposition is challenging and provocative and what more can be expected of hypotheses? It is perhaps unfortunate that the arrangement of the book precludes a unified consideration of growth both in higher and lower plants.

It is, however, a happy feature of his book that Prof. Roelofsen never hesitates to express his own opinion, for this builds what might have been to the general reader a dreary review into a well-knit whole, a fascinating easily read story.

The book is translated from an original manuscript in Dutch, and both the author and Prof. C. E. B. Bremerkamp, who assisted in the translation, are to be congratulated that it reads so easily. There are flaws in the English, but these are nowhere such as to mislead the reader, even the use of the word "bruto" on p. 173. The book is copiously illustrated by electron micrographs, all of them drawn from the published papers of the writers whose work is discussed; these are necessarily of uneven quality since some are drawn from earlier work and some refer to difficult material. There is a comprehensive bibliography of some 815 references, and both subject and author indexes as well as indexes of botanical and zoological names.

This is a book which no botanist—nor any technologist interested in cellulose fibres—can afford to ignore. It is a first-class reference book for the specialist, a fascinating unfolding story of research down the years for general readers, and an instructive, thought-provoking book for advanced students. To all these, it is recommended in the warmest terms.

R. D. Preston

A Biochemical Approach to Pathology

By M. J. R. Dawkins and Dr. K. R. Rees. Pp. vii+128. (London: Edward Arnold (Publishers), Ltd., 1959.) 18*s*. net.

AS the advance of knowledge invades the old border-lines between the sciences, pathology becomes increasingly concerned with the chemistry of the cell, considered in topographical detail as an organized system of mainly enzymatically promoted reactions. Morbid states formerly described only in anatomical and histological terms have now to be interpreted in terms of chemical derangement in cytoplasm, microsome or other specified site. The first assertion is the justification of this book; the second describes the approach taken by its authors. Within the confines of 120 pages, two disciplines are bridged with an adequacy necessarily dependent on wise selection and enforced abbreviation. The authors have succeeded so well in combating these limitations that there is ground for some regret that more space could not be made available to them. Whereas pathologists may find difficulty with the assumption of extensive prior reading in chemistry, biochemical readers are less severely taxed in pathology, but both are aided by a wisely chosen short guide to further reading. Some of the present frontiers of knowledge are indicated in a stimulating manner.

The unfortunate tendency to write in initials (RNA, DPN and too many others) is strongly maintained without benefit of a glossary and detracts from an otherwise eminently readable text, while important substances such as adenylic acid appear only by name, assuming the glib role of shibboleth.

For a first edition, this well-produced volume is commendably free from errors, excepting the trivalent carbon shown in the structure of bilirubin (p. 40) and the confusion of β-amino-α-naphthol with *o*-aminophenol (p. 115). R. W. R. BAKER

Proceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy held in Geneva, 1 September—13 September 1958

Vol. 2: Survey of Raw Material Resources. Pp. x+843. (Geneva: United Nations; London: H. M. Stationery Office, 1958.) 80 Swiss francs; 132*s*.; 18.50 dollars.

THE geological discussions at the second Geneva conference on atomic energy, reported in this volume, had a much more international character than those at the first conference in 1955. At the latter, the delegation from the United States contributed 85 out of 125 papers on geology and prospecting, Great Britain and Brazil each produced four contributions, and the remaining 32 works came from 22 different countries. In 1958 only 30 papers out of 84 were of American origin and there was a much larger presentation from other lands, notably from the U.S.S.R., France, Canada, South Africa and India. Discussions were also more open, for as a direct consequence of the Free World's glut of uranium no aspect of the economic geology of radioactive ores is now secret in the West—although information on the location and output of domestic deposits is still 'classified' in the U.S.S.R. The radiogeological studies printed in this "Survey of Raw Material Resources" range widely over the diverse fields of economic geology, prospecting, geochronology, uranium mineralogy, and geochemistry; and they make up what is undoubtedly the most comprehensive assemblage of new information on radioactive mineralization that has ever been brought together. C. F. DAVIDSON

Sechzig Jahre Medizinische Radiologie

Probleme und Empirie. Von Prof. Hans R. Schinz. Pp. 275. (Stuttgart: Georg Thieme Verlag, 1959.) 19·50 D.M.

THIS book is a historical document written by Prof. H. R. Schinz of Zurich on the occasion of the Ninth International Congress of Radiology in 1959. It covers 60 years of medical radiology from the date of discovery of X-rays up to the present. The first section of the book deals with the discovery of X-rays by Röntgen. This is preceded by a short annotation leading up to this momentous achievement. The discovery of uranium and radium and their radioactive properties by Becquerel and the Curies is dealt with in a similar way, and so is the fundamental early work on nuclear and radiation physics by Rutherford and his co-workers. This first chapter covers all the important milestones in experimental and theoretical physics up to the 1950's, listing all the great names connected with this work throughout the world.

In the second section there is a short historical note on technical achievements and the development of X-ray apparatus. The main section of the book deals with the medical aspects of radiology, in particular radiobiology, X-ray diagnosis and radiation therapy and their advances during the past 60 years. Finally, some of the original papers of the early days of radiation physics by Röntgen, Rutherford, Becquerel, the Curies, Hahn and Strassmann are appended.

This book is a useful historical review of the past 60 years, mentioning all the outstanding workers in the field: it also contains a number of interesting historical photographs and charts referring to the very early fundamental work. R. E. STEINER

How Animals Move

By Sir James Gray. (The Royal Institution Christmas Lectures 1951.) Pp. 144. (Pelican Book A454.) (Harmondsworth, Mddx.: Penguin Books, Ltd., 1959.) 3*s*. 6*d*.

IN 1951, Sir James Gray gave six Christmas lectures to a young audience at the Royal Institution. His task was ". . . to illustrate the more striking features of animal locomotion without assuming previous biological knowledge". The lectures, together with many admirable illustrations and revealing photographs, were published by the Cambridge University Press in 1953. It is good that the book will now be available to many more readers, who will discover an absorbing subject treated in clear and direct English. Young naturalists (and older ones) will learn that the movements of animals can be illusory or impossible to follow without suitable recording devices. Correct observations require much circumspection (for example, anglers should not be misled as to the speed of a hooked fish by the scream of a reel). Lastly, the reader, who could be an artist just as well as someone with an interest in biology, will find a lucid analysis of the living mechanics of locomotion, one that parallels the natural ease of the animals themselves. N. B. MARSHALL

PRIMARY STRUCTURE OF THE PROTEIN OF TOBACCO MOSAIC VIRUS

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THE tobacco mosaic virus, in spite of its limited economic importance, has continually played a noteworthy part in virology. It was the first infectious agent to be classified as a virus by the pioneering experiments of Iwanowski (1892) and Beijerinck (1898). It was also the first virus to be isolated in a pure paracrystalline state by Stanley in 1935 and to be identified as a nucleoprotein by Bawden and Pirie. The ribonucleic acid was isolated from tobacco mosaic virus and proved to be infectious by Gierer and Schramm[1] and by Fraenkel-Conrat and Singer[2]. Later, this experiment was successfully repeated with many other plant and animal viruses containing ribonucleic acid[3,4]. Thus we are led to the concept that ribonucleic acid is the genetic material of these viruses and determines the structure of the virus protein. Due to intense study in various laboratories the physical and chemical structure of tobacco mosaic virus is the best known of all viruses. Unfortunately, tobacco mosaic virus is less suitable for genetic studies; the bioassay is of limited sensitivity, single cells in culture media are not infected at a satisfactory rate, and combinations between different strains are not observed, making gene mapping impossible. But one can assume that tobacco mosaic virus will remain a favourite object for studies of the structural interrelationship between ribonucleic acid and its specific protein. One important step in this direction is the precise knowledge of the sequence of amino-acids in the protein.

Studies concerning the tobacco mosaic virus protein began shortly after its isolation, but the further progress, which depended upon the development of methods in protein chemistry, was slow. Using methods which now appear primeval, W. M. Stanley[5] and G. Schramm and H. Müller[6] isolated certain amino-acids by fractional crystallization. At that time the task of finding the complete amino-acid sequence in a particle with a molecular weight of 40×10^6 seemed quite hopeless. A solution of this problem seemed more possible after it was discovered that the virus protein consists of a great number of sub-units which appear identical in their physical and serological properties[7]. In weak alkaline solutions a dissociation into protein sub-units of a molecular weight of 90–100,000 was observed. This protein was called *A*-protein[8]. Later the virus protein could be dissociated further to the smallest sub-unit, which has a molecular weight of 17,500[9–11]. By re-aggregation of the *A*-protein[12] rod-like particles could be obtained which had a structure similar to the virus itself.

After development of chromatographical methods, especially paper chromatography, and the stimulating success of Sanger in the complete analysis of insulin (1949–55), the elucidation of the protein structure became experimentally possible. Using a combination of columns consisting of organic and inorganic adsorbents certain groups of amino-acids were estimated[13]. The first complete analysis was reported by Knight[14], who applied microbiological methods. More precise analyses became possible through the development of more reliable methods for the separation and quantitative estimation of amino-acids by ion exchange columns, a method for which we are indebted to Stein and Moore *et al.*

Further work of this group produced the automatic amino-acid analyser[15], without which the results described in this paper would have been virtually impossible. Cation-exchange columns have been extensively used for the separation and preparation of peptides. For the analyses of ribonuclease, Hirs, Stein and Moore[16] employed 'Dowex 50'. Braunitzer *et al.*[17] introduced an important modification by using the anion exchanger 'Dowex 1', with which the separation of peptides, particularly those containing basic amino-acids, was far more satisfactory. Using volatile buffer solutions containing pyridine and collidine, the laborious desalting process was avoided.

Before the analysis of the amino-acid sequence in a protein, the amino-acid composition and the number and nature of the terminal groups must be known. This is the most reliable way to prove the homogeneity of the material. The C-terminal group in the tobacco mosaic virus protein was identified by Harris and Knight[18] with carboxypeptidase. They found one mole of threonin per 17,300 gm. of tobacco mosaic virus. This value was confirmed by hydrazinolysis[19]. By short treatment with hydrazine the C-terminal peptide Pro-Ala-Thr was isolated[20,21], whereas by enzymatic hydrolysis Niu and Fraenkel-Conrat[22] determined the sequence of the last six amino-acids to be Thr-Ser-Gly-Pro-Ala-Thr. Since the virus does not contain a free terminal amino-group[23] the determination of the N-terminal configuration was more difficult. After treatment of the virus with trichloroacetic acid or hydroxylamine the imino-group of proline was released[24]. Therefore it was first assumed that proline, blocked by a labile substituent, was terminal. Later it was found that the Asp-Pro-bond inside the peptide chain is particularly labile and Pro could therefore not be N-terminal[25]. The correct N-terminal groups were found to be N-Acetyl-Ser-Tyr by Narita[26]. It was then clear that tobacco mosaic virus protein consists of about 2,200 peptide chains with the same terminal sequences.

It was then conceivable that the location of the other amino-acid residues in these peptide chains might also be identical. Therefore sequence studies were begun in several laboratories. Tryptic digestion of the protein was the first step, since the reaction of trypsin is quite specific, affecting only the carboxylic groups of the basic amino-acids. Because only a relatively small number of peptides is expected in the digestion mixture, the separation of these peptides ordinarily is not too difficult. In the Virus Laboratory in Berkeley a partial separation of the tryptic peptides, chiefly by counter-current distribu-

tion, was achieved and the sequence and composition of several of these tryptic peptides have already been described[27]. Wittmann and Braunitzer[28] separated all the tryptic peptides using 'Dowex-1' anion exchangers. They found twelve different peptides, accounting for all of the amino-acids. For the first time the amino-acid content of tobacco mosaic virus could be calculated exactly by summing up the composition of the peptides. According to these authors the peptide chain consists of 157 amino-acids and has a molecular weight of 17,400, which is in good agreement with the terminal group determinations and with physical, chemical and X-ray studies[9–11,18,19,21,29]. Furthermore, the N-terminal groups of eleven of these peptides were identified and the arrangement of several of these peptides inside the chain was elaborated by comparison of the tryptic peptides of different strains of tobacco mosaic virus[30].

Our own studies also began with the tryptic hydrolysis of the protein separated from the ribonucleic acid by extraction with phenol or acetic acid. In early experiments the separation of the peptide was tried by dinitrophenylation and subsequent paper electrophoresis and paper chromatography. The results were very unsatisfactory. Later we turned to column chromatography with the cation exchange resin 'Dowex 50' as the adsorbent. Most of the peaks arising in the effluent were mixtures of different peptides which had to be separated further by high-voltage electrophoresis and paper chromatography. Much better results were obtained with the anion exchange resin 'Dowex-1' according to the technique of Wittmann and Braunitzer. Only a few boundaries contained more than one peptide. While preparing the digest for column chromatography, we found a peptide insoluble in acidic solutions. This peptide was called *TU* (*tryptisch, unlöslich*). A similar peptide was observed by Gish *et al.*[27] and by Ramachandran *et al.*[27] and designated as I-peptide (insoluble). The *TU* peptide was purified by several precipitations between *p*H 3 and 5 and by paper chromatography. It contains 41 amino-acids and has the same composition as the peptide No. XII of Wittmann and Braunitzer. The N-terminal group is N-Acetyl-Ser-Tyr. Therefore *TU* is the N-terminal peptide of the whole chain. The sequence of the amino-acids in this peptide is described by Anderer, Weber and Uhlig[31]. Besides the *TU* peptide eleven other tryptic peptides were found. The amino-acid compositions of all were determined with the automatic amino-acid analyser. The N- and C-terminal groups were identified, and the sequential structure elaborated so far as necessary to correlate with peptic and chymotryptic peptides described below. In Table 1 the amino-acid composition and the terminal groups of the twelve tryptic peptides are indicated in the order in which they occur in the peptide chain. The analytical data agree exactly with those of Wittmann and Braunitzer. Summing up the tryptic peptides, the molar ratios of the amino-acids in the total chain can be calculated. In the order of their frequency we obtain the following formula:

$$Asp_{18}Glu_{16}Ser_{16}Thr_{16}Ala_{14}Val_{14}Leu_{12}Arg_{11}Pro_{8}Ileu_{8}$$
$$Phe_{8}Gly_{6}Tyr_{4}Try_{3}Lys_{3}Cys_{1}$$

Analysis of the total protein confirmed these ratios. The time of hydrolysis for the analyses was varied between 17 and 60 hr. and the values obtained in five experiments were extrapolated to zero time.

In order to find the sequence of the tryptic peptides the protein was degraded with chymotrypsin and pepsin. Attention was primarily directed to peptic and chymotryptic peptides which formed bridges between two tryptic peptides (bridge peptides). Since all tryptic peptides end with Arg or Lys the bridge peptides must contain these basic amino-acids. In certain experiments only the Arg-containing peptides were selected by the Sakaguchi reaction. The peptides were separated on columns of 'Dowex-50' or 'Dowex-1'. Further purification was achieved by paper chromatography of the dinitrophenyl derivatives. In this way about 80 different peptides were isolated and their amino-acid composition and sequence determined. The C-terminal sequence was studied by hydrolysis with carboxypeptidase *A*. If the terminal group was Arg or Lys, a mixture of carboxypeptidase *A* and *B* was employed. The N-terminal groups were mainly identified by the dinitrophenyl method. In order to find the second residue the terminal dinitrophenyl amino-acid was removed by hydrogenation according to the method of Justisz[32] or of Uhlig[33], and the dinitrophenylation was repeated. Occasionally the Edman degradation was also applied. Further insight into the N-terminal configuration was obtained by degradation with aminopeptidase. Sequence analysis was completed by partial hydrolysis with 0·1 *N* or 12 *N* hydrochloric acid or further digestion with chymotrypsin or pepsin. Of the 80 peptides only the 22 bridge peptides are given in Table 2. We have been able to isolate all bridges which were necessary to connect the twelve tryptic peptides in an unequivocal way and thus to deduce the structure of the entire chain of 157 amino-acids. The derivation of the formula can be understood by comparison of Tables 1 and 2 with Fig. 1.

The peptides I–III (Table 2) contain the C-terminal sequence of the *TU* peptide followed by Thr. Since only one tryptic peptide exists beginning with Thr, this must be adjacent to the *TU* peptide. The peptides IV–VII contain the C-terminal sequence of the Thr-peptide and the N-terminal sequence of the $GluNH_2$-peptide. Therefore, the $GluNH_2$-peptide occupies the positions 47–61. This $GluNH_2$-peptide contains one Lys residue which is resistant to trypsin. The peptides VIII–X demonstrate the sequence adjacent to this first Lys residue. The junction with the next peptide beginning with Phe and ending with Lys is given by the peptides XI and XII. The locations of the tripeptide Val-Tyr-Arg and the following peptide beginning with Tyr are deduced from the peptides XIII–XV. The peptic peptide XVI shows the location of the dipeptide $AspNH_2$-Arg and the following Ileu-peptide. This is joined to the next peptide by an Arg-Arg-bond (see peptides XVII and XVIII). The carboxylic group of the second Arg residue is attacked slowly by trypsin, thus the tryptic peptide position 112–121 contains Arg as the N-ter-

Table 1. Amino-acid Composition of Peptides obtained by Digestion of Tobacco Mosaic Virus Protein with Trypsin

Position	
1–41	Acetyl-Ser(Asp_4,Thr_4,Ser_4,Glu_4,Pro_2,Gly,Ala_4,Val, $Ileu_2$,Leu_4,Tyr,Phe_2,Cys,Try)Arg (ref. 31)
42–46	Thr(Val_2,Glu)Arg
47–61	$GluNH_2$(Thr,Ser_2,Glu_2,Pro_2,Val_2,Phe,Try,Lys)Arg
62–68	Phe(Asp_2,Ser,Pro,Phe)Lys
69–71	Val-Tyr-Arg
72–90	Tyr(Asp_2,Thr_2,Pro,Gly,Ala_2,Val_2,Leu_4,Phe)Arg
91–92	$AspNH_2$-Arg
93–111	Ileu(Asp_2,Thr_4,Glu_4,Pro,Ala_2,Val,Leu)Arg
112–121	Arg(Asp_2,Thr,Ala_2,Val_2,Ileu)Arg
122–133	Ser(Asp_2,Glu,Ala,Val,$Ileu_2$,Leu_2)Arg
134–140	Gly(Asp,Thr,Ser,Gly,Tyr)Arg
141–157	Ser(Thr,Ser_2,Glu,Pro,Gly_2,Ala,Val,Leu,Phe,Try)Thr

Fig. 1. Sequence of amino-acids in the protein of tobacco mosaic virus

minal group. This is followed by the tryptic peptide beginning with Ser-Ala, and the location of the last two peptides is given by the peptides XX–XXII. Additional evidence for the C-terminal position of the Ser-peptide (141–157) is that its terminal sequence Pro-Ala-Thr is identical with that found to be the C-terminal sequence of the tobacco mosaic virus protein. Only the relative positions of the residues 26–28 and 98–102, which are set in parentheses, need reinvestigation. The amidation of the Glu and Asp residues marked with an asterisk has not been determined. Apart from this couple of uncertainties a good picture of this large peptide is obtained.

Discussing the reliability of these results we have to keep in mind the following facts. The amino-acid composition of the tryptic peptides agrees completely with that found by Wittmann and Braunitzer and the sequence of the first five tryptic peptides has also been confirmed by Wittmann[20] in quite a different way. Some of the tryptic peptides are also described by the Berkeley group. Errors are further restricted because all the positions are covered by several degradation products obtained from the total protein or the isolated tryptic peptides. The complete list of all degradation peptides for the positions 1–41 has already been published[21] and that for the other positions will be published elsewhere. In Fig. 1 the points of cleavage by trypsin, chymotrypsin, and pepsin are indicated to give a general idea of the number of peptides which were laboriously separated and characterized.

Table 2. Sequences of Peptides containing Arg and Lys obtained by Digestion of Tobacco Mosaic Virus Protein with Chymotrypsin (*C*) and Pepsin (*P*)

No.	Enzyme	Position	Sequences
I	*P*	40–42	Ala-Arg-Thr
II	*P*	36–42	Glu*-Thr-Glu-GluNH$_2$-Ala-Arg-Thr
III	*P*	35–42	Phe-Glu*-Thr-Glu-GluNH$_2$-Ala-Arg-Thr
IV	*P*	43–50	Val-Glu*-Val-Arg-GluNH$_2$-Phe-Ser-GluNH$_2$
V	*P*	43–47	Val-Glu*-Val-Arg-GluNH$_2$
VI	*P*	45–47	Val-Arg-GluNH$_2$
VII	*C*	46–48	Arg-GluNH$_2$-Phe
VIII	*C*	53–59	Lys-Pro-Pro-Ser-GluNH$_2$-Val-Thr
IX	*C*	53–57	Lys-Pro-Pro-Ser-GluNH$_2$
X	*P*	51–59	Val-Try-Lys-Pro-Pro-Ser-GluNH$_2$-Val-Thr
XI	*P, C*	60–67	Val-Arg-Phe-Pro-Asp*-Ser-Asp-Phe
XII	*P*	60–66	Val-Arg-Phe-Pro-Asp*-Ser-Asp
XIII	*P*	68–75	Lys-Val-Tyr-Arg-Tyr-Asp*-Ala-Val
XIV	*C*	68–70	Lys-Val-Tyr
XV	*C*	71–72	Arg-Tyr
XVI	*P*	88–94	Asp*-Thr-Arg-AspNH$_2$-Arg-Ileu-Glu*
XVII	*P*	108–117	Asp*-Ala-Thr-Arg-Arg-Val-Asp*-Asp*-Ala-Thr
XVIII	*P*	108–119	Asp*-Ala-Thr-Arg-Arg-Val-Asp*-Asp*-Ala-Thr-Val-Ala
XIX	*P*	120–127	Ileu-Arg-Ser-Ala-Asp*-Ileu-AspNH$_2$-Leu-
XX	*P*	132–137	Ileu-Arg-Gly-Thr-Gly-Ser
XXI	*P*	132–143	Ileu-Arg-Gly-Thr-Gly-Ser-Tyr-AspNH$_2$-Arg-Ser-Ser-Phe
XXII	*P, C*	139–143	AspNH$_2$-Arg-Ser-Ser-Phe

Asp* = Asp or AspNH$_2$; Glu* = Glu or GluNH$_2$

The careful analysis of larger peptide chains furnishes several kinds of information. Proteins have specific functions and their biosynthesis is under genetic control. Therefore we cannot expect a random sequence of amino-acids. The accumulation of Ser in the first 15 and in the last 17 positions is striking. These terminal parts, comprising 20 per cent of the molecule, contain 11 of the 16 Ser residues. Of course, more data on other proteins must be collected before any conclusion can be drawn about regularities in the combinations of individual amino-acids.

Furthermore the results reveal information about the specificity of the proteinases. Trypsin was strictly specific for the basic amino-acids; only the Lys residue in position 53 was resistant, perhaps due to the adjacent Pro, and the Arg residue in position 112 was inhibited. A Lys residue resistant to trypsin was also found in ribonuclease[35]. Chymotrypsin liberated the carboxylic group of Phe, Tyr, Try, and Leu, and less frequently that of GluNH$_2$ (three times) and of Thr (twice). Pepsin reacts rather unspecifically. It frequently affects the carboxylic or the amino group of Phe, Tyr, Leu, and Val. Ileu and GluNH$_2$ were also attacked in a few cases.

Since the ribonucleic acid of tobacco mosaic virus induces the synthesis of the protein a relationship

between the sequences of the nucleotides and amino-acids, respectively, must be assumed. The ribonucleic acid contains about 6,000 nucleotides, that is, 40 nucleotides per 1 amino-acid residue in the protein. But are so many nucleotides actually required to bring one amino-acid into the right position? The determination of the entire sequence of 6,000 nucleotides is beyond the limits of our present experimental techniques. However, it may be possible to find certain correlations utilizing the inadequate technique at present available. By treatment with nitrous acid the nucleotide pattern of the ribonucleic acid can be altered *in vitro* and genetically stable mutants are produced[38,39]. The amino-acid sequences of these mutants should be compared with that of the normal strain to find out whether all mutants have undergone an alteration in the protein. If the ribonucleic acid produces other proteins, the genetical change could occur in these and the virus protein remains unaffected. In this case the ratio of nucleotides per amino-acid would be less than 40.

Fig. 1 shows only the primary structure of the tobacco mosaic virus protein. In the virus particles this chain is folded into an ordered secondary structure leading to protein sub-units. These protein sub-units are arranged in a helix which we call the tertiary structure. By denaturation the tertiary and secondary structures are destroyed, but under certain conditions the disordered protein can be re-naturated and re-aggregated to the original tertiary structure[36]. By reconstitution[37] with ribonucleic acid the tertiary structure is stabilized and the length of the protein helix is adapted to the length of the nucleotide chain. The importance of the amino-acid sequence in determining the internal structure of the tobacco mosaic virus particle is therefore evident.

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RADIOACTIVE FALLOUT DURING 1959

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THE suspension of nuclear weapons testing from November 1958 until February 1960 has provided a useful period during which measurements of the relative abundance of different radionuclides in fallout material may be used to obtain some indication of the average age of this material and its atmospheric history. Further interest attaches to this period, for, as shown recently[1], the concentration of strontium-90 in rain falling over Great Britain rose sharply during the first half of 1959 to values substantially higher than those found for previous years. We have made measurements of the radiostrontium content of rain-water, sampled throughout 1959, and have also studied the variations in the concentration of certain short-lived fission products present in surface air during the same period. The results of these investigations are presented here and are discussed briefly in relation to current theories on the transport of fission products in the atmosphere.

Rain-water samples were collected at a site near Leatherhead, Surrey, using a polythene funnel and collecting bottle (funnel area for early samples, 325 sq. cm., and latterly, 1,320 sq. cm.). Radiostrontium determinations were made following well-established methods[2]. Samples of atmospheric dust were collected from surface air (of the order of 10^7 l.) at our laboratory at Sutton, Surrey, using a large two-stage electrostatic precipitator developed originally for

Table 1. Principal Nuclides 1 yr. after Fission showing their Main Modes of Decay*

Nuclide	Half-life of: Parent	Half-life of: Daughter	Maximum β-energy for principal mode of decay (MeV.)	Principal γ lines: Energy (MeV.)	Principal γ lines: Transitions per 100 disintegrations	Disintegrations/min./10^{19} fissions† after 1 yr. for: ^{235}U (thermal)	^{235}U (fast)	^{239}Pu (thermal)
^{137}Cs ^{137m}Ba	30 yr.	2·6 m.	0·5	0·66	83	25	25	20
^{90}Sr ^{90}Y	28 yr.	64 h.	0·6 2·2			20	10	‡
^{147}Pm	2·6 yr.		0·2			90	‡	110
^{106}Ru ^{106}Rh	370 d.	30 s.	0·04 3·5	0·51 0·62	21 10	20	140	250
^{144}Ce ^{144}Pr	285 d.	17 m.	0·3 3·0	0·13	~ 5	350	320	350
^{95}Zr	65 d.		0·4	0·76 0·72	49 49	90	80	85
^{95}Nb		35 d.	0·2	0·77	100	180	160	170
^{91}Y	58 d.		1·5			60	‡	30
^{89}Sr	53 d.		1·5			30	20	10
^{103}Ru	40 d.		0·2	0·50 0·61	90 7	30	70	60

* Based on information cited in refs. 6, 7 and 8.

† Except in the case of (zirconium-95 + niobium-95), it may be assumed that equal amounts of parent and daughter nuclides are present at equilibrium. The figures shown opposite nuclide pairs give the values for the parent only.

‡ Information not available.

air-cleansing purposes[3] (air-flow, 3,800 l./min.; efficiency of precipitation for methylene blue particles, 0·5μ mass median diameter, 80 per cent). These samples were studied by γ-scintillation spectrometry using a 100-channel analyser[2].

Samples collected during May–September 1959, that is, more than six months after the last reported nuclear weapons test in 1958, showed three prominent photopeaks. These peaks were at about 0·14, 0·50 and 0·75 MeV., which correspond to the γ-energies of cerium-144, (ruthenium-103 + rhodium-106) and (zirconium-95 + niobium-95) respectively. These are the main γ-emitting nuclides to be expected in a 6–18-month old mixture of fission products (Table 1). We have therefore assumed that these were the nuclides measured. A number of checks on the decay of their activity at various times after collection provided further confirmation of the validity of this assumption. The activities presented in Table 4 were computed from the areas under the photopeaks, using an experimentally determined calibration and making due allowance for the known branching ratios of the nuclides (Table 1, column 6). An estimate of the separate contributions of ruthenium-103 and rhodium-106 to the 0·51-MeV. peak was derived from two sets of measurements, one made shortly after collection and the other after a period covering several life-times of ruthenium-103.

Table 2. Quarterly Concentration (10^{-12} curie/litre) of Strontium-90 in Rain, 1959

Quarter:	1st	2nd	3rd	4th
Activity:	13·3	15·8	4·6	1·0

The average concentrations of strontium-90 found by us in rain-water collected over quarterly periods during 1959 are shown in Table 2. The values for the first two quarters are similar in magnitude to those reported recently[1], being approximately three times the average values found in the same periods of the previous four years[4]. The marked reduction of activity in the third and fourth quarters is illustrated in detail by monthly values from May onwards. These are plotted on a logarithmic scale against time in Fig. 1 (upper curve, μμc./l.).

It is well established[5] that, for long-range fallout of nuclear debris injected originally into the stratosphere, the principal mechanism of deposition is wash-out in rain-water after entry into the troposphere. The amount deposited by dry turbulent deposition is on the average only about 3 per cent of the total. In consequence, measurements of the specific activity of strontium-90 in rain, for average rainfall conditions, provide a useful guide to the

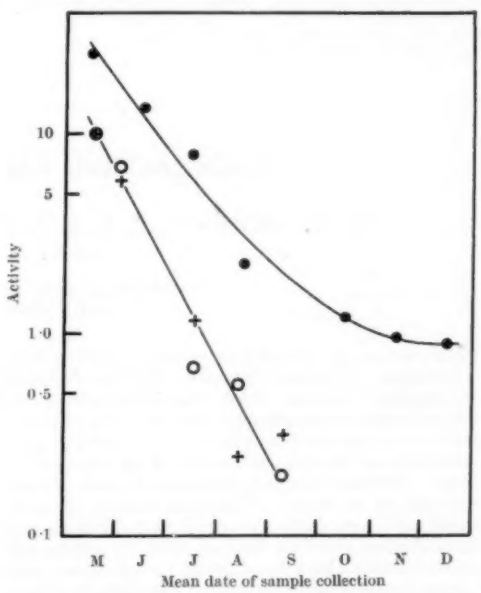

Fig. 1. Fission products in rain-water and air, 1959. ●, strontium-90 in rain; ○, rhodium-106 in air; + (zirconium-95 + niobium-95) in air

variations of activity in the lower atmosphere. It should be noted that the present monthly values for strontium-90 (Fig. 1) will include different fractional contributions from the dry deposition process, depending on the extent of departure from average conditions. Thus, from the known rainfall, and following the previous findings of Stewart *et al.*[5], it appears that for both May and June, which had comparatively low rainfalls, a dry deposition contribution of about 20 per cent should be allowed, compared with about 5 per cent for the other months, which had near average conditions. With due allowance for this factor, the results indicate that the concentration of strontium-90 in the lower atmosphere at the end of 1959 had fallen to one-twentieth of that present in May of the same year. Since the amount of radioactive decay of strontium-90 during this time would be negligible, the rapid fall in activity is clearly due to meteorological factors.

Table 3. STRONTIUM-89/STRONTIUM-90 RATIOS IN RAIN, 1959

Month :	May	June	July	August	Sept.	Oct.
$^{89}Sr/^{90}Sr$*	7·3	5·5	2·6	2·7	—	< 1·0

* ^{89}Sr ($T_{1/2}$ = 51 days) values calculated to mid-month.

The strontium-89/strontium-90 ratios of the rainwater (Table 3) provide a measure of the age of the strontium-90 being deposited during the summer of 1959. These ratios may be compared with the maximum values to be expected on the assumptions of (1) a strontium-89/strontium-90 ratio between 150 and 200 at the time of fission[6,7] and (2) an absence of weapons testing since November 1958. This comparison leads to the conclusion that on the average 70–85 per cent of the strontium-90 deposited during May–August 1959 was derived from fission processes which occurred during the autumn of 1958.

The results obtained for the concentrations of rhodium-106 and (zirconium-95 + niobium-95) in surface air are shown in Table 4. Compared with strontium-90, these radionuclides are short-lived, and during the period of the investigation a substantial radioactive decay of the atmospheric reservoir of these activities must have occurred. Using the known half-lives of these nuclides, the values in Table 4 may be re-scaled to eliminate the effect of the radioactive decay which occurred after the date of the first sample (May 17, 1959). The values adjusted in this way have been plotted on a logarithmic scale in arbitrary units against time (Fig. 1, lower curve). The two sets of results (rhodium-106 and zirconium-95 + niobium-95) have been normalized after equating the values for the first sample.

It is useful to compare the two curves plotted in Fig. 1, since both reflect changes in the radioactive particle content of the lower atmosphere. It should be noted, first of all, that our measurements relate to the atmosphere at a single site. However, since a prolonged period for atmospheric mixing elapsed between the last reported test in 1958 and the start of the present investigation, it is clear that the results represent conditions in the atmosphere over a comparatively wide area. This conclusion is underlined by the results of Stewart *et al.*[5] on the spread of nuclear dust clouds which were formed in the lower atmosphere from low-yield explosions. These workers showed that the lateral spread was virtually complete after a few weeks. Thereafter, apparently as the result of removal by deposition, the specific nuclear dust content of the air decreased exponentially, the 'half-life' for this process being approximately 22 days.

Table 4. CONCENTRATIONS (10^{-17} CURIE/LITRE) OF RHODIUM-106 AND (ZIRCONIUM-95 + NIOBIUM-95) IN AIR

Mean date of collection	Period of collection (days)	Rhodium-106*	(Zirconium-95 + niobium-95)
17. 5.59	4	17·0	180
2. 6.59	11	11·3	88
16. 7.59	16	1·02	11
12. 8.59	13	0·79	1·8
8. 9.59	11	0·25	1·7
31.12.59	21	0·26	0·25

* The ratio ruthenium-103/rhodium-106 for the sample at 17.5.59 was estimated to be 0·96.

Looking at Fig. 1, it will be seen that the values plotted for rhodium-106 and (zirconium-95 + niobium-95) lie fairly close to a straight line. This indicates that the concentration in air of fallout particles, which contained these nuclides, decreased exponentially during May–September 1959. The slope of the line shows that the 'half-life' of the removal process was approximately 20 days. The close similarity of this figure to that noted above for the removal of nuclear dust distributed in the lower atmosphere indicates that during June–September 1959 there were no substantial additions of relatively short-lived fission products to the lower atmosphere, either from a stratospheric reservoir, or from fresh weapons tests.

It will also be seen from Fig. 1 that the trend of the values of strontium-90 plotted for May–August 1959, is similar to that for the shorter-lived materials. The slope of the curve until August suggests a 'half-life' for removal of strontium-90 of between 30 and 40 days. Thereafter, the apparent rate of removal becomes greatly reduced and approaches zero at the end of the year. This is a clear indication that at the end of 1959 strontium-90 levels were being maintained by material from a stratospheric reservoir.

Taken together, the various lines of evidence presented above lead to the picture that about 75 per cent of the fallout of strontium-90 during the summer of 1959 was derived from a tropospheric reservoir built up during the early months of the year from material injected into the upper atmosphere in the autumn of 1958. Towards the end of 1959 it appears that the strontium-90 levels were then maintained largely by material descending from the stratosphere.

The average age of the strontium-90 in the stratospheric reservoir is a question of some interest. A comparison of the current trend of levels of relatively short-lived fission products with that of strontium-90 throws light on this question. It should be noted that the estimation of rhodium-106 in air was complicated at the end of 1959 by the fact that beryllium-7 produced by cosmic rays (γ line, 0·48 MeV.) was present in the air at about the same concentration as the fission-product nuclide (results in preparation). The values presented for rhodium-106 and (zirconium-95 + niobium-95) at December 1959 (Table 4) have been estimated by γ-spectrometry with due allowance for the effect of beryllium-7 on the rhodium-106 photopeak. After correction for radioactive decay, these December values suggest that the lower curve of Fig. 1 should be continued approximately parallel to the upper curve. This indicates that a large proportion of the fallout at the end of 1959 originated in fission processes which occurred during 1958.

During the years 1955–58 the trend of strontium-90 concentration in rain falling over Great Britain has

shown fairly regular seasonal fluctuations, with maxima in the spring and minima in the autumn, the ratio of maximum to minimum being on the average 4 (ref. 1). These fluctuations have been interpreted by Stewart[5] as representing a seasonal variation in the rate of descent of stratospheric air, carrying long-lived fission products, into the troposphere. Martell[10] has advanced an alternative explanation suggesting that each maximum has been due to a rapid descent of activity in the spring from tests conducted at high latitudes in the autumn of the preceding year, the minimum values representing levels due to a more or less steady leak from a relatively persistent stratospheric reservoir. The results obtained by us for 1959 are clearly consistent with Martell's suggestion. It should be noted, however, that the strontium-90 maximum occurring in 1959 was notably higher than in previous years, and that the ratios strontium-89/strontium-90 do not exclude the possible occurrence of older stratospheric material at levels corresponding in magnitude to previous maxima. Consequently, it is not clear whether the increase in the maximum value for 1959, compared with previous maxima, reflects simply a quantitative difference, or whether two different upward fluctuations have been superimposed. In the latter case, it might be expected that the levels of activity would show a rise early in 1960. In this connexion it is interesting to record that the value found by us for the concentration of strontium-90 in rain collected during January 1960 was 1·7 μμc./l., which is approximately twice the value for December 1959. A preliminary analysis of our early 1960 dust samples also indicates an upward trend of rhodium-106 concentration in the air.

It may be useful to note that the nuclear test conducted on February 13, 1960, was carried out at a period when the activity already being deposited was apparently increasing. It is clear, therefore, that any upward trend in total atmospheric activity appearing after mid-February 1960 would not necessarily indicate a rise due to recent weapons testing. The effect of the French test (February 13) at the present site was to produce a pronounced, but transitory, rise of activity, comprising mainly short-lived fission products[11].

We wish to thank our colleague, Mr. R. P. Parker, for valuable information on the relative abundance of rhodium-106 and beryllium-7 in recent dust samples.

We are indebted to Prof. W. V. Mayneord, director of the Physics Department, Institute of Cancer Research: Royal Cancer Hospital, for his guidance and encouragement throughout this work.

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DENSITY OF THE UPPER ATMOSPHERE AND ITS DEPENDENCE ON THE SUN, AS REVEALED BY SATELLITE ORBITS

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In previous articles in *Nature*[1-3] on upper-air density, a method of finding density from changes in the orbits of artificial satellites was presented, values of average density were obtained from fifteen satellites launched before September 1959, and the variation of density with latitude and season was discussed. The present article extends these results, giving revised values for air density at heights between 180 and 700 km. deduced from twenty-one satellites at dates up to March 1960, and describes two significant new features of the upper atmosphere which have now become apparent.

Method of Analysis

A satellite moving in an elliptic orbit encounters most air drag when it is closest to the Earth, and so the air density in this region, at heights near that of perigee, can be determined from the rate of contraction of the orbit, if the mass, size and shape of the satellite are known.

The method of analysis adopted here is the same as before[3]. The air density ρ is assumed to vary exponentially with height y, so that

$$\rho = \text{const.} \times \exp(-y/H) \qquad (1)$$

where H is a constant, the value of which is not known exactly[3]. It can then be shown[1] that, if H^* is the best estimate of H, the air density ρ^*, say, at a height $\frac{1}{2}H^*$ above perigee height is given in terms of the rate of change of orbital period, dT/dt, by the equation:

$$\rho^* = -\frac{0{\cdot}158}{\delta}\frac{dT}{dt}\sqrt{\frac{e}{aH^*}}\left\{1 - 2e - \frac{H^*}{8ae} + 0\left(e^2, \frac{H^2}{a^2e^2}\right)\right\} \qquad (2)$$

where a is the semi-major axis of the orbit, e its eccentricity, and δ a drag parameter, discussed in the next paragraph. If $0{\cdot}02 < e < 0{\cdot}15$, as for most satellites, and if, as is almost certain, H^* does not differ from the true value of H by a factor of more than 1·5, the maximum error in equation (2) is less than 5 per cent. If e is increased to 0·2, the maximum error rises to 10 per cent. If $e < 0{\cdot}02$,

Table 1. VALUES OF $\delta = FSC/m$ FOR SATELLITES 1957α–1959λ

Satellite		Mass m (kgm.)	m/SC_D (kgm./sq. m.)	δ (sq. m./kgm.)
Sputnik 1	1957α2	83·6	110	0·0088
Sputnik 1 rocket	1957α1	—	63	0·015
Sputnik 2	1957β	—	62	0·015
Explorer 1	1958α	14·0	23	0·039
Vanguard 1	1958β2	1·47	23	0·040
Vanguard 1 rocket	1958β1	22·7	18	0·050
Explorer 3	1958γ	14·1	23	0·039
Sputnik 3	1958δ2	1,327	210	0·0046
Sputnik 3 rocket	1958δ1	—	63	0·015
Explorer 4	1958ϵ	17·5	29	0·032
Atlas	1958ζ	3,960	28	0·032
Vanguard 2	1959α1	9·75	20	0·044
Vanguard 2 rocket	1959α2	22·7	18	0·050
Discoverer 2	1959γ	635	48	0·021
Discoverer 5	1959ϵ1	635	48	0·021
Discoverer 5 capsule	1959ϵ2	136	120	0·0081
Discoverer 6	1959ζ	635	48	0·021
Vanguard 3	1959η	45·4	26	0·035
Explorer 7	1959ι1	41·5	45	0·021
Explorer 7 rocket	1959ι2	5·9	19	0·048
Discoverer 7	1959κ	635	48	0·021
Discoverer 8	1959λ	635	48	0·021

equation (2) is replaced by a more generalized formula[3] in terms of Bessel functions.

The parameter δ in equation (2) denotes the quantity FSC_D/m, where F is a factor which allows for rotation of the atmosphere and lies between 0·9 and 1, S is the effective cross-sectional area of the satellite perpendicular to the direction of motion, C_D is a drag coefficient the value of which normally lies between 2 and 2·3, and m is the mass of the satellite. The value of S is, as before[2], calculated on the assumption that each satellite has rotated about an axis of maximum moment of inertia, which may, however, have any direction in space, so that S is taken as the mean of the values in the two extreme forms of motion—travelling like an aeroplane propeller, and tumbling end-over-end. The value of C_D is, in accordance with the results of Cook[4] for free-molecule flow with diffuse reflexion, taken as 2·2 for the spherical and nearly cylindrical satellites, and 2·15 for the conical *Sputnik* 3. Values of δ for *Sputnik* 2 and the rockets of *Sputniks* 1 and 3, for which m and S are unknown, are found by comparison with *Sputniks* 1 and 2, as explained previously[1].

Table 1 lists the values of δ thus obtained for all the satellites launched before March 1960 and having known orbits, apart from *Explorer* 6 (1959δ) and *Lunik* 3 (1959θ), to which the theory is not applicable, since e is much greater than 0·2. The values for the *Discoverers* refer to the period after the ejection of their capsules. The error (standard deviation) in the tabulated values of δ is estimated to be rather less than 10 per cent.

Day-to-Night Variations in Density

At heights of 200–300 km. the density probably does not depart from its average value by more than 10 per cent as a result of day-to-night variation[3,5], but at heights above 400 km. this day-to-night variation becomes increasingly important. For *Vanguard* 1, which has a perigee height of about 650 km., the perigee point first entered the Earth's shadow about July 1959, and at about the same time the slope of the period-versus-time curve was greatly reduced. This effect was noted by Jacchia[5] and further examined by Martin and Priester[6,19]. Since then, five other satellites with perigee heights between 550 and 650 km. have exhibited a similar effect, as Fig. 1 strikingly demonstrates. Fig. 1 shows the change in the orbital period during the life-times of the satellites *Vanguard* 1, *Vanguard* 2 and *Explorer* 7, and their rockets (1958β2 and 1, 1959α1 and 2, and 1959ι1 and 2: perigee heights 650, 560 and 560 km., respectively). Each curve is drawn as a full line when perigee is in sunlight, and as a broken line when perigee is in the Earth's shadow. For all six satellites the slope of the period-versus-time curves shows a gradual change from a maximum value in day-time to a minimum value at night; with the three satellites for which well-defined maximum and minimum slopes are available, the values differ by a factor of about 8. This shows that the drag acting on the satellites, and hence probably the air density, is much greater by day than by night at these altitudes. For *Explorer* 1 (perigee height 350 km.) a similar but much smaller variation has been noted[5].

Results

Fig. 2 shows the values of air density obtained by applying the method of analysis already described to twenty-one of the satellites listed in Table 1. The required orbital elements are based on the Table of Artificial Satellites issued by the Royal Aircraft Establishment, Farnborough. In this table values for the *Sputniks* are from British observations and theory[7]; those for the American satellites are compiled from data issued by the U.S. prediction centres, and from theory. Values of H^* for use in equation (2) are taken from a smoothed version of the values of H previously recommended[2]. At heights below 300 km. no attempt is made to separate day-time and night-time values of density; but for satellites with perigee higher than 300 km., two values of dT/dt are distinguished, the maximum day-time value and the minimum night-time value. These have for convenience been labelled 'midday' and 'midnight' values in Fig. 2; but they represent rather the average density during the time, usually lasting several weeks, when the satellite's perigee is in the 'early afternoon' position where density appears to be highest[8] (or in the 'after midnight' position where density is least). The resulting values of density are less accurate than those at lower altitudes, with a standard deviation of perhaps ± 20 per cent above 400 km., and the curves are therefore drawn as broken lines in Fig. 2.

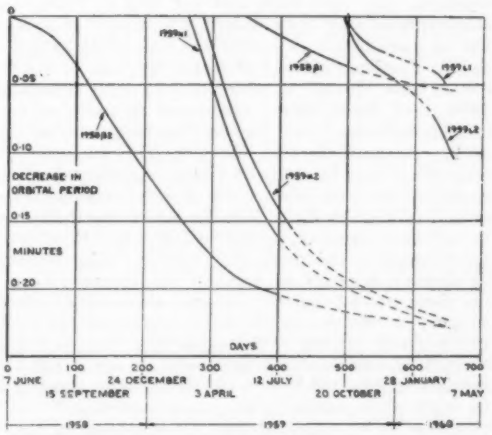

Fig. 1. Decrease in orbital period for *Vanguards* 1 and 2, *Explorer* 7 and their rockets
——— perigee in sunlight; – – – perigee in Earth's shadow

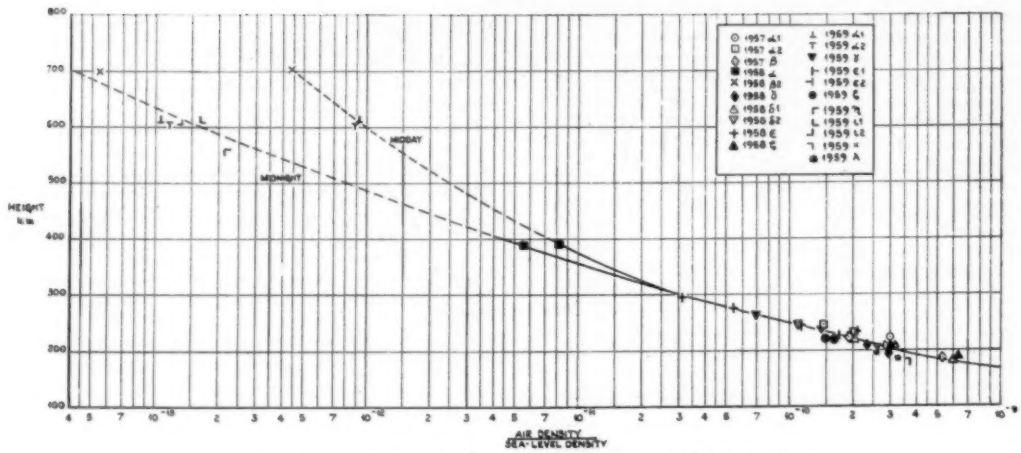

Fig. 2. Density of the upper atmosphere obtained from the orbits of 21 satellites

In discussing the features of interest in Fig. 2, it is best to begin at the lower altitudes. At heights between 180 and 300 km. there are 27 points, referring to satellites of different sizes and shapes, at different seasons, latitudes, etc.: 22 of the points differ from the curve by a factor of less than 1·35 and none by a factor of more than 1·6. This confirms previous evidence[1-3] that the density did not depart from the long-term average for 1957–59 by a factor of more than 1·5 as a result of variations with latitude or season, or from day to night, though there might be variations of larger amplitude during time intervals too short for the orbits to register, that is, less than one day.

A second significant feature of Fig. 2, not apparent at first sight, is a slow variation of density with time at heights of 180–250 km. The 24 points in this region can be divided into two groups, for October 1957–January 1959 (14 points) and for August 1959–March 1960 (10 points), respectively, and the mean curves through each of these groups give densities which are respectively about 10 per cent higher and 10 per cent lower than the curve of Fig. 2. Thus, there has apparently been a small decrease in average density between, say, mid-1958 and the end of 1959, and this explains why the points in Fig. 2 are not so near the average curve as in the corresponding graph of ref. 2. Further evidence of such a decrease in density comes from *Sputnik* 3, which was in orbit from May 1958 until April 1960: the usual methods of predicting life-time have, for this satellite, consistently under-estimated the true life-time. This unexpectedly long life would result from either a general decrease in density or a reduction in the effective cross-sectional area S of the satellite; but the latter possibility can be ruled out because, for *Sputnik* 3, S is little affected by changes in the mode of rotation[4]. The decrease in density appears to confirm the suggestion in a previous article[3] that the average air density is likely to vary in the course of the 11-year sunspot cycle. For it is well known[5-12] that solar disturbances largely control the day-to-day variations in air density at heights of 200–300 km., with the density tending to be high when sunspots are most numerous[13]. It is therefore to be expected that, as indicated by Fig. 2, the average air density will be highest at the sunspot maximum, in 1957–58, and will decrease slowly for a few years thereafter.

For heights between 300 and 700 km. the results in Fig. 2 from the various satellites agree well, and the midday and midnight values are clearly distinguished. The good agreement may be partly fortuitous, since for some of these satellites dT/dt is not well defined, being based on very small changes in period. The density given by the 'midday' curve in Fig. 2 exceeds that on the 'midnight' curve by factors of 1·6, 3, 6 and 11 at heights of 400, 500, 600 and 700 km., respectively. It should be remembered that the density changes gradually between its daytime maximum and night-time minimum, as Fig. 1 shows: the density seems to depend primarily on the zenith distance of the Sun, density being highest when the Sun is nearly overhead[6]. Thus, at heights above 500 km., the density should appear to vary with latitude and season; for example, density would be high by day in the equatorial regions and low near the poles in winter. But it would be misleading to call such effects 'variation with latitude', since such a nomenclature would tacitly attribute to terrestrial geometry an effect whose true source is solar.

At heights of 400–700 km. the major uncertainty in the values of density is, as has been emphasized before[1,2], the role of electrical drag. As a satellite moves through the ionosphere it tends to collect electrical charge, most probably negative charge. The potential to which the satellite becomes raised is not usually known, however, and, even if it were, the magnetohydrodynamic theory of the drag of a charged body in these conditions has not been properly formulated. The potential was estimated by Jastrow and Pearse[14] to be about − 10 volts in darkness, while measurements on *Sputnik* 3 indicated potentials between − 2 and − 6 volts in sunlight[15]. If the potential is of order − 10 volts, the theory of Kraus[16] suggests that at heights of 600–700 km. the electrical drag would be less than 10 per cent of the total drag, and would thus scarcely be significant in considering a change of drag by a factor of 8. At present, therefore, it appears most probable that the changes in density indicated in Fig. 2 are real; but, if the potential were much higher than assumed above, the electrical drag might have an important effect.

Another possible source of error is the effect of the radiation pressure P of sunlight. An approximate

Table 2. Values of Air Density and H given by the Curves of Fig. 2

Height (km.)	Air density / Sea-level density		Density (gm./c.c.)		H (km.)	
180	$5{\cdot}7\times10^{-10}$		$7{\cdot}0\times10^{-13}$		30	
190	$4{\cdot}2\times10^{-10}$		$5{\cdot}1\times10^{-13}$		36	
200	$3{\cdot}2\times10^{-10}$		$3{\cdot}9\times10^{-13}$		43	
210	$2{\cdot}6\times10^{-10}$		$3{\cdot}2\times10^{-13}$		47	
220	$2{\cdot}1\times10^{-10}$		$2{\cdot}5\times10^{-13}$		44	
230	$1{\cdot}6\times10^{-10}$		$2{\cdot}0\times10^{-13}$		42	
240	$1{\cdot}3\times10^{-10}$		$1{\cdot}5\times10^{-13}$		41	
250	$9{\cdot}8\times10^{-11}$		$1{\cdot}2\times10^{-13}$		40	
260	$7{\cdot}7\times10^{-11}$		$9{\cdot}4\times10^{-14}$		41	
270	$5{\cdot}9\times10^{-11}$		$7{\cdot}2\times10^{-14}$		42	
280	$4{\cdot}7\times10^{-11}$		$5{\cdot}7\times10^{-14}$		44	
290	$3{\cdot}8\times10^{-11}$		$4{\cdot}6\times10^{-14}$		47	
300	$3{\cdot}0\times10^{-11}$		$3{\cdot}7\times10^{-14}$		50	
	day	night	day	night	day	night
300	$3{\cdot}0\times10^{-11}$	$3{\cdot}0\times10^{-11}$	$3{\cdot}7\times10^{-14}$	$3{\cdot}7\times10^{-14}$	50	50
350	$1{\cdot}4\times10^{-11}$	$1{\cdot}1\times10^{-11}$	$1{\cdot}7\times10^{-14}$	$1{\cdot}4\times10^{-14}$	74	53
400	$7{\cdot}4\times10^{-12}$	$4{\cdot}5\times10^{-12}$	$9{\cdot}1\times10^{-15}$	$5{\cdot}5\times10^{-15}$	83	56
450	$4{\cdot}2\times10^{-12}$	$1{\cdot}9\times10^{-12}$	$5{\cdot}1\times10^{-15}$	$2{\cdot}3\times10^{-15}$	90	59
500	$2{\cdot}5\times10^{-12}$	$8{\cdot}2\times10^{-13}$	$3{\cdot}1\times10^{-15}$	$1{\cdot}0\times10^{-15}$	99	62
550	$1{\cdot}5\times10^{-12}$	$3{\cdot}6\times10^{-13}$	$1{\cdot}9\times10^{-15}$	$4{\cdot}4\times10^{-16}$	109	65
600	$1{\cdot}0\times10^{-12}$	$1{\cdot}6\times10^{-13}$	$1{\cdot}2\times10^{-15}$	$2{\cdot}0\times10^{-16}$	118	69
650	$6{\cdot}6\times10^{-13}$	$8{\cdot}2\times10^{-14}$	$8{\cdot}1\times10^{-16}$	$1{\cdot}0\times10^{-16}$	126	73
700	$4{\cdot}5\times10^{-13}$	$4{\cdot}2\times10^{-14}$	$5{\cdot}5\times10^{-16}$	$5{\cdot}1\times10^{-17}$	133	77

Sea-level density taken as 0·001226 gm./c.c.

analysis suggests that the change per revolution in the orbital period T of a satellite of reflectivity ε is of the order :

$$\frac{3SPT}{mg}(1+\varepsilon)\left(\frac{a}{r_2}-\frac{a}{r_1}\right)$$

where r_1 and r_2 are the distances of the satellite from the Earth's centre when it (1) enters and (2) emerges from the Earth's shadow, and g is the average gravitational acceleration while the satellite is in eclipse. In the long run, this effect will tend to cancel out, since the mean value of the second bracket is zero, though for a satellite with perigee remaining in sunlight for a long time—such as *Vanguard* 1—the effect builds up ; even for this satellite, however, the change in the orbital period in six months would be only about 0·001 min., which, as Fig. 1 shows, is less than 10 per cent of the total change, even when the perigee is in darkness. The effect is likely to be important only for an inflated-balloon satellite with a perigee which is arranged to remain in darkness for several years.

To sum up, it appears likely that at heights between 200 and 300 km. the curve of Fig. 2 gives the average density for 1957–60 with a standard deviation of about 10 per cent, though the density has decreased by perhaps 20 per cent between 1958 and 1960. At heights above 400 km. the curves become less accurate, and it should be noted that, by chance, the midday values all refer to early 1959 and the midnight values to late 1959 or early 1960, so that any general decrease in density during this time, to match the decrease at lower altitudes, will have widened the apparent difference between day and night.

The curves of Fig. 2 agree well with the direct measurements of air density made by the manometers in *Sputnik* 3, as reported by Michnevich *et al.*[17]. At heights between 225 and 300 km. the difference is less than 20 per cent ; at heights of 300–500 km. the direct measurements fall between the two curves of Fig. 2, within the region which would be expected from the local time of the measurements. Also the density at a height of 430 km., obtained from the expansion of a sodium cloud from a Russian Geophysical Rocket in September 1958 (ref. 18), is within 5 per cent of the midday curve in Fig. 2.

Values from the curves of Fig. 2 are listed in Table 2.

Scale Height and Air Temperature

The values of the coefficient H of equation (1), which is nearly equal to the atmospheric scale height[1], can be found from the slope of the density-versus-height curves of Fig. 2, and values are listed in the last column of Table 2. There is a slight indentation in the curve of Fig. 2 at a height near 210 km., though this is less pronounced than in the corresponding curve in ref. 2, because the scatter of the points is greater. The values of H in Table 2 are subject to maximum errors of about $\pm$ 20 per cent between 180 and 300 km. height and to larger errors at greater heights.

The value of air temperature can be deduced[1] from H and the mean molecular weight of the air, M, which is, however, not known exactly. Taking $M = 22$ at 200 km., decreasing to $M = 16$ at 500 km., and constant thereafter, and using the values of H from Table 2, the temperature obtained at a height of 200 km. is about 1,200° K. ; at a height of 600 km. the value would be about 1,200° K. in darkness, or about 2,200° K. in sunlight.

Conclusions

The wide variation of density between midnight and midday at heights above 500 km., which had previously been noted for one satellite[5], has now shown itself for five other satellites (see Fig. 1), and the day-time and night-time values revealed by each conform to a consistent pattern (see Fig. 2).

At heights between 180 and 250 km., the average air density seems to have decreased appreciably, by perhaps 20 per cent, between 1958 and 1960. Since it is known that day-to-day variations in air density depend on solar activity, this change is interpreted as the beginning of an 11-year cycle, maximum density occurring at the sunspot maximum in 1957–58. At heights above 250 km. this effect ought to be more prominent.

We are grateful to the United States prediction centres, Project Space Track and the Smithsonian Astrophysical Observatory for supplying many of the values of orbital period used in this article.

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OBITUARIES

Prof. J. H. C. Whitehead, F.R.S.

Prof. J. H. C. Whitehead, who died suddenly in Princeton on May 8, was one of our greatest mathematicians. His contributions to topology were both massive and fundamental. He also had an international reputation as a geometer and as an algebraist. In research he went from strength to strength, uniting profound study with ever-growing creativity.

Henry Whitehead, as he was known, was Waynflete professor of pure mathematics in Oxford. He lived in the Otmoor village of Noke where his wife Barbara runs a farm, in which he took the liveliest interest. Their exuberant hospitality brought many and varied friends to Manor Farm, as it had done previously to their house in north Oxford. They shared great zest for life and enjoyed a marriage of surpassing happiness. They have two sons, David and Oliver.

Whitehead was born in India in 1905. His father, Bishop of Madras and brother of the philosopher A. N. Whitehead, sent him to Eton, a school for which he always had the highest regard. He went on to read mathematics at Balliol under H. W. Nicholson. After graduating, he first tried financial work in London but soon decided on a university career.

In 1928 he went to Princeton for three years as a Commonwealth Fellow to study under Oswald Veblen, whose high standards deeply influenced his mathematical thought and style. Together they wrote the important Cambridge Tract, "The Foundations of Differential Geometry", which is now regarded as a classic, and Whitehead also wrote a number of papers on differential geometry, including his Princeton Ph.D. thesis. Although he never lost his interest in geometry, it quickly became dominated by the study of topology, to which he was initiated by Veblen, Alexander and others. This young subject had run into formidable problems which have only recently begun to give way. The attempt to solve these strongly developed Whitehead's conceptual powers and led him towards some of his finest work.

On his return to Oxford as Tutorial Fellow at Balliol, he plunged into college life with huge enjoyment. He was an inspiring and open-hearted teacher, to whom mathematics mattered and the clock did not. He was an entertaining and resourceful talker with a highly independent view of people and affairs. He loved the more sociable kinds of sport, perhaps cricket most of all. In the Alps he did some fine climbs with Alexander, such as the Zmutt ridge of the Matterhorn and the Grand Charmoz, and he was also a good skier.

Following his marriage in 1934 Whitehead began to publish those pioneer studies which have made him famous. These were mostly on topological subjects, but there are also papers dealing with Lie algebras and free groups. By the beginning of the Second World War he was already concentrating on homotopy theory more than classical topology, and this remained his main interest until two years ago. The fifty papers he wrote on this subject have had great influence on its development and contain some of its most important theorems and ideas.

During the War, Whitehead worked for the Admiralty and the Foreign Office, but his research still continued. He was elected to the Royal Society and then, at the end of the War, he was chosen to succeed Dixon as Waynflete professor at Oxford. This made him a Fellow of Magdalen, where he continued to enjoy many aspects of college life. He never lacked students to inspire with his great knowledge and love of mathematics. Always generous with time and ideas, he managed to be encouraging while at the same time maintaining the highest standards. Students came to him from all over the world. To him is largely due the quality of the work which many of them have since done.

At Oxford Whitehead did much to foster the new Mathematical Institute, with its friendly atmosphere and spirit of hard work. He helped to found the very successful series of British Mathematical Colloquia. Through this and through the London Mathematical Society the invigorating effect of his personality was felt far outside Oxford. He valued international contacts and kept in close touch throughout with topologists in the United States, France, Poland, the U.S.S.R. and elsewhere.

Last year he felt ready to return, with vastly increased powers, to those problems which had challenged him as a student. Accordingly, he went on sabbatical leave to the United States, and it was there in Princeton that he died. I. M. James

Dr. C. S. Gum

On April 28, Dr. Colin Stanley Gum, one of Australia's outstanding younger generation astronomers, was killed in a skiing accident at Zermatt, Switzerland. He had just been appointed to the post of senior lecturer in astrophysics in the University of Sydney, and his visit to Europe was in connexion with plans for the installation of a reflecting telescope by the University.

Colin Gum was born in 1924. He graduated with honours in physics at the University of Adelaide in 1949 and proceeded to Mount Stromlo Observatory, where, in 1955, he obtained his Ph.D. degree in astronomy, the first given by the Australian National University. After a short period on the staff of the University of Adelaide, he joined the Commonwealth Scientific and Industrial Research Organization Radiophysics Laboratory in 1956, and in 1959 he was awarded a Carnegie fellowship to the Mount Wilson and Palomar Observatories, where he was working immediately prior to the fatal trip to Europe.

His work alternated between radio and optical astronomy but was consistently concerned with interstellar matter. His main contribution in the optical field was a major survey of hydrogen emission regions in the southern sky. In the Radiophysics Laboratory he studied the distribution in the Galaxy of interstellar hydrogen, using 21-cm. radio observations. Through this work he became a key member of the International Astronomical Union Commission which set up a new system of galactic co-ordinates in 1958, primarily on the basis of such information. At Mount Wilson and Palomar he had begun to work on interstellar polarization in external galaxies.

His death is deeply regretted by his colleagues in Australia, both radio and optical, and is a very serious loss to Australian science.

J. L. Pawsey

NEWS and VIEWS

Queen's Birthday Honours List

THE following names of scientists and others associated with scientific work appear in the Queen's Birthday honours list:

K.C.B.: Frank C. Francis, director and principal librarian, British Museum.

K.B.E.: Dr. Robert Cockburn, chief scientist, Ministry of Aviation.

Knights: Dr. Robert S. Aitken, vice-chancellor of the University of Birmingham; Dr. Basil F. J. Schonland, director, Research Group, U.K. Atomic Energy Authority; Dr. Gordon B. B. M. Sutherland, director of the National Physical Laboratory; Dr. Henry G. Thornton, foreign secretary of the Royal Society; Prof. Robert D. Watt, emeritus professor of agriculture in the University of Sydney.

C.B.: Dr. F. M. Lea, director of the Building Research Station, Department of Scientific and Industrial Research; Dr. J. S. McPetrie, director-general of Electronics Research and Development, Ministry of Aviation.

C.M.G.: N. F. Mitchell, president of the Royal Agricultural Society of Victoria; J. H. Parry, principal of University College, Ibadan, Western Region, Nigeria.

C.B.E.: Dr. T. E. Allibone, director of the Research Laboratory, Associated Electrical Industries, Ltd.; Prof. N. S. Bayliss, professor of chemistry in the University of Western Australia; L. Farrer-Brown, director of the Nuffield Foundation; J. S. Carter, chief alkali inspector, Ministry of Housing and Local Government; A. P. S. Forbes, director of agriculture, Tanganyika; Capt. G. I. D. Hutcheson, for services to the engineering profession in Australia; Prof. T. G. H. Jones, professor of chemistry in the University of Queensland; W. J. Langford, headmaster of Battersea Grammar School; R. B. McConnell, director of geological surveys, British Guiana; K. D. S. MacOwan, director of veterinary services, Kenya; Prof. M. E. L. Mallowan, professor of Western Asiatic archæology, University of London, and director of the British School of Archæology; H. F. Mooney, forestry and soil conservation adviser, Middle East Development Division, Lebanon; Prof. A. W. Scott, professor of chemical engineering, Royal College of Science and Technology, Glasgow, for contributions to the food dehydration industry and services to the Aberdeen research establishment of the Ministry of Agriculture, Fisheries and Food; A. F. M. Smith, director of lands and surveys, Uganda; Dr. R. A. Smith, chief scientific officer, Royal Radar Establishment, Ministry of Aviation; J. C. C. Stewart, deputy managing director, Production Group, U.S. Atomic Energy Authority; Dr. F. R. Tubbs, director of the East Malling Research Station, Maidstone; Dr. E. I. White, keeper of palæontology, British Museum (Natural History).

I.S.O.: P. A. Allison, lately conservator of forests, Western Region, Nigeria; T. Lacey, principal in the Department of Scientific and Industrial Research; E. W. Pratt, senior principal scientific officer, Admiralty.

Atomic Energy Research Establishment Appointments: Mr. B. S. Smith

MR. B. S. SMITH has been appointed head of the Isotope Research Division of the Atomic Energy Research Establishment. This Division was formed from the Isotopes Division as part of the re-organization of isotope production and application work carried out by the Atomic Energy Authority last year. The Division has recently completed a move from Harwell to the Wantage Research Laboratory, six miles away, which was opened by the Minister for Science, Lord Hailsham, on May 16. Mr. Smith is a graduate of King's College, London, where he was a demonstrator in physics from 1934 until 1939. During the War he worked at the Radar Research and Development Establishment, first on gun-laying radars and later on 'Identification, Friend or Foe Systems'. In 1944 he transferred to the Tube Alloy Project, and went to Canada, where he worked on the instrumentation for the Canadian *NRX* reactor. He returned to Harwell in 1947, and shortly afterwards joined the newly formed Health Physics Division. He became deputy head of the Division in 1954, and head when Dr. W. G. Marley took over the Radiological Protection Division of the Authority's Health and Safety Branch.

Mr. N. G. Stewart

MR. SMITH'S place as head of the Health Physics Division will be taken by Mr. N. G. Stewart, who is at present head of the Health and Safety Division at the Dounreay Experimental Reactor Establishment. Mr. Stewart graduated at the University of Aberdeen in 1937, where he gained first-class honours in mathematics and natural philosophy. After a period teaching, he joined the Radar Research and Development Establishment at the beginning of the War, and became leader of the Aerial and Waveguide Systems Section. He joined the Health Physics Division at Harwell in 1948, and became head of the Radiation Measurements Group. He was transferred to Dounreay in 1958.

Chemical Engineering at Nottingham: Prof. R. Edgeworth Johnstone

DR. ROBERT EDGEWORTH JOHNSTONE, at present assistant technical manager of Springfield Works, United Kingdom Atomic Energy Authority, has been appointed to be the first Lady Trent professor of chemical engineering in the University of Nottingham. The chair has been endowed by Boots Pure Drug Co., Ltd., Nottingham, in recognition of the services rendered to the University by the late Lord Trent and Lady Trent. Dr. Edgeworth Johnstone was educated at Wellington College and the Royal Military Academy, Woolwich, after which he spent two years at the Manchester College of Technology and a further year at University College, London. He graduated with first-class honours in applied chemistry and was awarded the degree of M.Sc. in chemical engineering of the University of London. In 1958, he was awarded the degree of doctor of science. He has had wide

experience in Great Britain and abroad of large-scale plant management and development, and after the War was design engineer for important new petrochemical and chemical units in Lancashire and London. For almost ten years he has been with the Ministry of Supply with special responsibility for pilot plant experiments and process studies, and he has published numerous articles on the problems of translating experimental and small-scale production methods into full-scale commercial schemes.

Research in Optical Design at the Imperial College, London

An optical design group is to be set up at the Imperial College of Science and Technology, London, under the direction of Dr. C. G. Wynne, of the Wray Optical Co., Ltd., with the support of a grant for an initial period of seven years of £9,400 a year from the Department of Scientific and Industrial Research. The group, initially employing a staff of four in addition to Dr. Wynne, will be established under Prof. W. D. Wright, professor of technical optics in the College. The scientific programme will be: (1) to develop new methods of lens design, including the use of electronic digital computers, and to give instruction in these methods; (2) to design and construct lens systems of orthodox type but improved performance; of novel types including systems employing aspheric surfaces; and of specialized systems required in very limited numbers but having unusual performance characteristics, such as for space research.

Dr. C. G. Wynne

Dr. Wynne graduated at Exeter College, Oxford, to which he won an open science scholarship in 1930. He joined Messrs. Taylor, Taylor and Hobson, Ltd., where he worked on lens design. In 1943 he became chief lens designer to Messrs. Wray (Optical Works), Ltd., and in 1949 a director of Wray (Developments), Ltd., a subsidiary company which has undertaken a considerable volume of new optical design. In 1955 he became technical director of Wray (Optical Works), Ltd. In 1953 he was awarded a Ph.D. by the University of London. Dr. Wynne is the honorary business secretary of the Physical Society and is the honorary secretary of the newly established Institute of Physics and the Physical Society. He has acted as English language editor of *Optica Acta* since its inception in 1954.

Forest Products Research Laboratory: Mr. A. C. Copisarow

Mr. H. Wooldridge, deputy director, Stations Division, in the Department of Scientific and Industrial Research, has agreed to withdraw his acceptance of the directorship of Forest Products Research (see *Nature*, January 23, p. 212) at the special request of the secretary of the Department, because of the increasing pressure of work at headquarters of the Department. Mr. A. C. Copisarow, at present scientific attaché at Paris, has been appointed director of the Forest Products Research Laboratory in succession to Dr. F. Y. Henderson, who retires in October. Mr. Copisarow graduated in 1942 in the honours school of geology at the University of Manchester. He served in the Royal Navy, for four years on radar duties, and during 1946–54 was with the Joint Intelligence Bureau of the Ministry of Defence. He has published papers on the colloidal chemistry of silica. A former Council of Europe Research Fellow, he has served at the British Embassy in Paris for six years.

The United States Geological Survey: Dr. W. H. Bradley

In appreciation of the work of Dr. 'Bill' Bradley, chief geologist of the United States Geological Survey during 1944–59, the *Americal Journal of Science* has published a 'Festschrift' of 38 papers (*Amer. J. Sci.*, Bradley Volume, **258–A**. Pp. 433 + 10 pl.; 1960) ranging over many topics in the Earth sciences. Dr. Bradley joined the U.S. Geological Survey as a 'geologic aid' in 1921, and soon became well known for his distinguished work on the oil shales and other strata of the lacustrine Green River Formation of middle Eocene age. These studies were interrupted by the War, when he was appointed chief of the Military Geology Unit responsible for supplying geological information to the American forces; and immediately afterwards he took over the leadership of the Geologic Division of the U.S. Geological Survey, which expanded under his charge from 400 geologists in 1944 to close on 1,000 in 1955. Recently he relinquished these administrative responsibilities to return to his initial research work. The volume in his honour is a fitting tribute to many years of good management of the largest geological organization in the Western world.

Advisory Committee on Poisonous Substances used in Agriculture and Food Storage

Sir Charles Dodds, Courtauld professor of biochemistry in the University of London, and director of the Courtauld Institute of Biochemistry at the Middlesex Hospital, has been appointed chairman of the inter-departmental Advisory Committee on Poisonous Substances used in Agriculture and Food Storage, in succession to Sir Solly Zuckerman. This Committee was recently reconstituted to include the following five members from outside the Government service; Prof. J. H. Gaddum, director of the Institute of Animal Physiology, Cambridge; Prof. Andrew Wilson, professor of pharmacology, University of Liverpool; Dr. J. Hamence, public analyst and agricultural analyst; Mr. F. H. Garner, principal of the Royal Agricultural College, Cirencester; and Prof. A. R. Clapham, professor of botany, University of Sheffield. The Committee was appointed in March 1954 to keep under review all risks that may arise from the use of toxic substances on agricultural products and in the storage of food, and to make recommendations to the Ministers concerned.

National Reference Library of Science and Invention

Answering a question regarding progress with the proposed National Reference Library of Science and Invention in the House of Lords on May 31, the Minister for Science said it is proposed to house this Library in a new Patent Office building to be erected on the South Bank, London. The terms of a lease of the site are now being settled between the Ministry of Works and the London County Council, and it is hoped to start building in the spring of 1963 with the view of completion by the end of 1965. Lord Hailsham had stated on July 30, 1958, that the Library would be built up and expanded from the existing Patent Office Library. He now said that it has been decided that it would be in the long-term interest of learning and of the completeness of the national collections of scientific literature for the National

Reference Library of Science and Invention to be formed as part of the British Museum Library, although housed as a separate unit. Scientific publications at present in the British Museum Library would be incorporated in the new Library. Care would be taken to safeguard the interest and convenience of those who use the present Patent Office Library, and they would be represented on an Advisory Committee to be set up when the new Library is brought into operation. Viscount de L'Isle welcomed this decision on behalf of the Trustees of the British Museum, and said that it would in due course make available to students and research workers the existing scientific resources of both the Patent Office and of the British Museum scientific collections in a compendious way and with the minimum of duplication.

State Scholarships and Commonwealth Co-operation

In the House of Lords on June 1, Lord Tweedsmuir asked the Government whether it would consider permitting up to 100 of the 2,000 State scholarships annually awarded in Britain to be tenable at Canadian or other Commonwealth universities if the recipient so elected. Lord Hailsham, replying for the Government, said that the Government would consider the matter in the light of the recommendations of the Anderson Committee. Asked by Lord Dalton to accelerate consideration of this question in view of the opportunities which Canadian and Australian universities can offer, Lord Hailsham agreed that it is a separate question from most of the contents of the Anderson Report, but thought that consultation with the Commonwealth Governments would be necessary before he could reply. Lord Casey suggested that the success of the proposal would depend on reciprocity between Commonwealth countries and the United Kingdom, and that into this consideration of cost and of available places would enter.

Preservation of North Devon Countryside

An area of the North Devon countryside about 66 square miles in extent is to be protected by the confirmation by the Minister of Housing and Local Government of an order made by the National Parks Commission. The area includes the whole of the Hartland Peninsula and such places as Clovelly, Braunton Burrows, Croyde, Woolacombe and Combe Martin, near the western edge of the Exmoor National Park. The order, which is made by the National Parks Commission under the National Parks and Access to the Countryside Act, 1949, designates these parts of North Devon as "Areas of Outstanding Natural Beauty", not as a National Park. The responsibility for preserving and enhancing the natural beauty of the landscape rests with the local planning authority, Devon County Council. Government grants can be made at the rate of 75 per cent towards the cost of treating derelict land, tree planting and preservation, and removing disfigurements.

Nature Conservation in Africa

Answering questions about Nature conservation in the Colonial territories in the House of Commons on June 2, the Secretary of State for the Colonies, Mr. Iain Macleod, welcomed the opportunity to reaffirm the Government's concern for wild life in Africa. Wild life conservation measures are, he said, continuously under review in the East and Central African territories, and in the past decade much has been done to establish and consolidate national parks and reserves. More use could be made of technical knowledge in developing the wild-life reserves, and the voluntary co-operation of the African populations is essential. The International Union for the Conservation of Nature, in consultation with other international agencies, is exploring the possibility of convening next year in Africa a conference on conservation. Mr. Macleod said that in a few weeks he would be able to study a detailed report by Dr. E. B. Worthington on his recent visit to Africa and the need for further help.

Journal of Child Psychology and Psychiatry

A new journal which will be generally welcomed has been issued by the Association of Child Psychology and Psychiatry. It is the *Journal of Child Psychology and Psychiatry and Allied Disciplines*, and is published under a distinguished editorial board of international authorities. The joint editors consist of a research psychologist, psychiatric social worker and a well-known physician in child-psychiatry, Dr. Emanuel Miller, while the corresponding editors are Prof. Milton Senn, Department of Pediatrics, Yale University, and P. H. Mursen, Department of Psychology, University of California. The *Journal* is attractively produced and the first issue (Vol. 1, No. 1 (January 1960). Pp. 98. Published quarterly. Subscription rates: To members of the Association of Child Psychology and Psychiatry 63*s*. per annum, inclusive of membership. To private individuals 105*s*. (15 dollars) per annum. To libraries and institutions 140*s*. (20 dollars) per volume. London and New York: Pergamon Press, 1960) contains articles which are refreshingly free from the jargon so beloved by students of human behaviour. The standard is set in the first article by Dr. Miller, who traces the origins of child-psychiatry, while Prof. Leo. Karner (Johns Hopkins University) shows that behaviour symptoms do not always indicate psychopathology; the clarity and brevity of his writing has much to commend itself to his fellow-American psychiatrists. Other articles deal with the effects of early deprivation and separation on children, manic-depressive psychosis in childhood, a gap in the structure of general psychology and a description of the training provided in child-psychotherapy at the Tavistock Clinic. Provided that the new journal can maintain this standard and keep itself free from psychiatric expertize, it could prove as useful to teachers and intelligent parents as to the professional child-psychiatrist and psychologist for whom it is intended.

Pavlov Journal of Higher Nervous Activity

This journal has been added to the list of Russian journals which are being published in English translation. By arrangement with the Soviet authorities, 'rush' copies of the original journal and its illustrations are to be made available, so that the delay in publication of the translation can be made as short as possible. The first issue of the 1959 volume contains 18 original communications, together with a short review of work carried out in the United States of America during the years 1954–57 on conditioned reflexes in animals. There is an obituary of the eminent Russian physiologist, L. A. Orbeli (Vol. 9, No. 1. Translated from the Russian. Pp. 140. Published bi-monthly. Approximately 1,000 pages per volume. Annual subscription 210*s*.; 30 dollars. London and New York: Pergamon Press, 1960).

Medical and Biological Compilation

The second edition of "Current Indexing and Abstracting Periodicals in the Medical and Biological Sciences" attempts to provide an annotated list of all the most important indexing and abstracting periodicals in medicine and the allied sciences published currently throughout the world, and the number of titles has been increased from 142 in the first (1953) edition to 310 (World Health Organization. Library News, Vol. 12, Supplement 2. Pp. 85. Geneva: World Health Organization, 1959). In general annual publications containing review articles are excluded, but the yearbook series, containing the abstracts, are included. Periodicals are listed alphabetically by title, with the name and address of the publisher and/or responsible organization, first year of publication, current subscription price, year and volume number of the first volume in the World Health Organization holding, frequency of publication, number of periodicals regularly scrutinized and of abstracts or title entries published annually.

Transvaal Museum

This active museum publishes both a *Bulletin* and *Annals*. The former for January 1960, No. 4, includes some notes on mammal specimens as museum material and the collecting expedition of Colonel Jack Scott to Somalia. A short article also describes the bird-life at Sandwich Harbour. The April issue of the *Annals* (14, Part 1; 1960) contains an important key to the genera of South African freshwater and estuarine gastropods, observations on the locomotion of the South African adder, *Bitis peringueyi*, and on the early post-natal development of multi-mammate mice. The dibatag, *Ammodorcas clarkei*, is also dealt with in an important illustrated paper by Mr. J. Meester.

Psychiatric Services and Architecture

A booklet published by the World Health Organization attempts an analysis, not only of the planning and management of psychiatric hospitals, but also of the structure and function of other psychiatric services such as out-patients' departments, psychiatric wards in general hospitals, rehabilitation facilities, and other mental health services (Public Health Papers, No. 1: Psychiatric Services and Architecture. By A. Baker, R. Llewelyn Davies and P. Sivadon. Pp. 59. Geneva: World Health Organization; London: H.M. Stationery Office, 1959. 2 Swiss francs; 3*s.* 6*d.*). It is the joint work of two psychiatrists and an architect who has specialized in hospital architecture, but it also takes account of the comments and suggestions of twenty-nine other psychiatrists and four architects from other countries. It represents an international approach to the problem of how psychiatric services can best be organized in the interests of the patients and be given suitable architectural expression. The types of buildings proposed are very different from the gloomy, prison-like mansions which until recently were regarded in many parts of the world as the only appropriate places in which to incarcerate mental patients; the approach to the patient is based upon the desire to re-integrate him into society with all possible speed, and to provide him with surroundings that will contribute to that end.

Roe Deer

Two of Britain's native deer, the red and the roe, have been known to inhabit the country since pre-historic times. The roe is now more widely distributed in England than the red, but, in Scotland, the red deer is the more widespread species. There are few, if any, in Wales, and none in Ireland. Because there are no predatory animals to keep these deer in reasonable numbers, the burden of doing this rests on the owners of the land on which they live. As the planted forests grow taller the problem of control will increase, and it is important that game-keepers and foresters should be familiar with their habits. To help them, the Forestry Commission has produced another of its attractive illustrated booklets, on the roe deer, which would be equally useful for natural history lessons in schools (Leaflet No. 45: The Roe Deer. Pp. 16. (London: H.M. Stationery Office, 1960.) 1*s.* 6*d.* net). It describes not only methods of control but also how these attractive animals may be stalked, the terms of venery, the calls of the roe, and, not least attractive, a suggested method of cooking roast haunch of roe.

A New British Transistorized Computer

Through the joint enterprise of the General Electric Company and International Computers and Tabulators, Ltd., 149 Park Lane, London, W.1, a new, fully transistorized, automatic digital computer, the 1301, has been produced. This machine has characteristics which place it in the front rank of the large British computers which are available at the present time. It has storage, both on magnetic cores and on drums, the former in units of 400 words; the latter in units of 12,000 words; magnetic tape transports and a high-speed line-at-a-time printer are also available. The arithmetical speeds of the machine are such that addition and like operations take about 25 μsec., and multiplication about eight times as much. These fast operating speeds are achieved with the remarkably low pulse-operating time of 1 Mc./s.; this is apparently due to the unusual feature of the machine, namely, that it operates in serio-parallel decimal code, each decimal digit being represented by signals on four lines in parallel and each computer word consisting of twelve decimal digits. Probably the most surprising feature of the machine is its low power consumption, which the makers state to be only 4 kW. In common with most recently produced machines, a comprehensive range of sub-routines and automatic programming facilities is also available. Because it is built with magnetic tape as a standard and planned-for ancillary, this machine is more attractive than many of the British machines of the immediate past, in which the later addition of magnetic tape has only been achieved by considerable alterations.

Rock Pressure and Earthquakes

Whereas the virgin pressure of the fluids in rock formations at depth can be measured readily, the pressure in the solid rock itself could not, until recently, be measured directly. In practical problems concerned with mining and deep drilling it has been customary to assume that the pressure is satisfactorily defined by the weight of overburden. A method of direct measurement has been developed in Sweden which shows that this assumption is too simple. The method and results are described by Nils Hast in a recent issue of *Sveriges Geologiska Undersökning* (Ser. C, No. 560: The Measurement of Rock Pressure in Mines. By Nils Hast. Pp. 183. Stockholm: Sveriges Geologiska Undersökning, 1958).

The principle is to pre-stress a small stress-measuring cell situated in a hole drilled some distance into the rock, and then to cut away a portion of the surrounding rock by drilling an annular hole co-axial with and enclosing the first. The stress in the core of rock containing the cell is thus removed, and the difference between the original and final load measured by the cell gives the absolute value of the stress acting in the rock at the measuring point. The cell measures stress in one direction only; by using three boreholes the stress ellipsoid may be determined.

This method was first tried in 1951 in the Grängesberg iron mines, and has since been improved and applied to other mines. It has been found to be of great value in investigating the stability of roofs, walls and pillars, and the use of block caving as an efficient means of ore extraction. Besides their immediate practical value, however, the measurements promise to throw new light on the dynamics of the Earth's crust. In all cases where rock pressures have been measured in Sweden there has been found a pronounced excess of the horizontal component, usually 1·5–3·5 times the amount accounted for by the weight of overburden. These large horizontal stresses are ascribed by Hast to secular movements in the crust. Since many earthquakes are believed to be due to the sudden failure of parts of the crust following a steady accumulation of stress, it is further suggested that periodic measurements of rock pressure in earthquake regions might disclose the stage reached in the preliminary build-up, leading to the possibility of earthquake prediction.

The Wellcome Trust: Grant to the Massachusetts General Hospital, Boston

THE Wellcome Trustees have made a grant of £50,000 to the Massachusetts General Hospital, Boston, for the establishment of a Henry S. Wellcome research chair in medical science. The professor will be chosen by a special committee of the Massachusetts General Hospital, Harvard University, and the Massachusetts Institute of Technology, from a number of workers who have already achieved a high scientific reputation for their researches. The appointment will be made for ten years. This is the largest single grant to be made by the Wellcome Trustees to a medical research centre in the United States. In 1956 they made a grant of nearly £25,000 to the University of Pennsylvania to provide a Wellcome associate research professorship of anesthesiology for five years, and earlier in the present year they made a grant of £15,000 to construct a laboratory for cardiovascular research within the Department of Medicine at Johns Hopkins Hospital, Baltimore.

The Waverley Gold Medal Competition

THE Scientific Advisory Board of *Research* has decided to continue the Waverley Gold Medal essay competition, and this year will be the eighth year in succession in which it is offered. The Waverley Gold Medal, named after the late Lord Waverley, together with £100 will be awarded for the best essay of about 3,000 words describing a new scientific project or practical development, giving an outline of the background, the experimental results and the potential application of the project or process in industry. A second prize of £50 will be awarded, and also a special prize of £50 for the best entry from a competitor under the age of thirty on July 31. Further information can be obtained from the Editor of *Research*, 4/5 Bell Yard, London, W.C.2. The last date of entry is July 31.

Teratology Society

FOLLOWING the fourth Teratology Conference, which was held at the Memorial Sloan-Kettering Cancer Center in New York City and attended by 76 scientists from Canada, England, France, Germany and the United States, the Teratology Society was formed. One of the main purposes of the Society is to hold regular meetings at which investigations concerned with etiology and morphogenesis of congenital malformation can be presented and discussed. The following officers were elected: *President*, Dr. Josef Warkany (Cincinnati); *President-elect*, Dr. James G. Wilson (Gainesville); *Secretary-Treasurer*, Dr. Marjorie M. Nelson (San Francisco); *Recorder*, Dr. Sidney Q. Cohlan (New York); *Council*, Dr. F. Clarke Fraser (Montreal), Dr. David L. Gunberg (Portland) and Dr. M. Lois Murphy (New York). The National Foundation assisted in the formation of the Society with advice and financial aid. Further information concerning the society can be obtained from Dr. Marjorie M. Nelson, Department of Anatomy, School of Medicine, University of California, San Francisco, 22.

The Agricultural Society of India

THE following have been elected members of the new Executive Council for 1960 of the Agricultural Society of India: *President*, Dr. J. N. Mukherjee; *Vice-president*, Sri Bimal Chandra Sinha; *Editor*, Dr. P. K. Sen; *Secretary-treasurer*, Sri. R. M. Datta; *Other members*, Dr. N. R. Dhar, Dr. J. C. Sen Gupta, Dr. B. C. Kundu, Dr. H. K. Nandi, Dr. M. C. Das, Dr. S. K. Mukherjee, Dr. T. M. Das, Dr. U. N. Chatterjee, Dr. D. N. Roy Choudhury, Sri R. S. Roy, Sri T. K. Bose.

University News: Birmingham

DR. S. MANDELSTAM, assistant research physicist, Department of Physics, University of California, has been appointed to the newly established chair of mathematical physics within the Department of Mathematical Physics from October 1. The following lectureships are also announced: Dr. R. W. Whitworth (physics), Dr. A. B. Robbins (physics), Dr. J. R. Majer (chemistry), Dr. E. H. Creaser (senior lecturer in microbiology), Dr. F. W. J. Teale (biochemistry), and Dr. D. G. Walker (biochemistry). Research fellowships are as follows: A. M. Hoare (pure mathematics), J. S. Lilley (nuclear physics), B. Cockayne (William Gibbins Research Fellow in the Department of Physical Metallurgy) and E. N. Corlett, senior Research Fellow in the Department of Engineering Production).

Bristol

APPOINTMENTS to lectureships are as follows: Dr. P. G. Drazin (mathematics), Dr. A. E. Scott (chemistry), Mr. J. K. Whitaker (mathematical economics). Mr. K. F. H. Murrell has been appointed a Research Fellow in the Department of Psychology. Dr. D. H. Perkins has been elected reader in physics.

Durham

THE following appointments to lectureships are announced: Dr. O. E. W. R. Kurth (senior lecturer in mathematics), Mr. D. J. Bellamy (botany), Dr. R. D. Chambers (chemistry), Dr. D. P. Hendry

(psychology), Dr. D. M. Hirst (geochemistry), Dr. M. J. Morant (applied physics).

London

Dr. W. Klyne, reader in biochemistry at the Postgraduate Medical School of London, has been appointed to the University chair of chemistry tenable at Westfield College; and Dr. R. E. O. Williams, deputy director of the Central Public Health Laboratory, Colindale, has been appointed to the University chair of bacteriology tenable at St. Mary's Hospital Medical School. Appointments to readerships are as follows: Dr. J. H. Green (physiology), tenable at Middlesex Hospital Medical School; Dr. H. J. J. Blackwood (oral anatomy) at the Royal Dental Hospital of London School of Dental Surgery; J. C. de C. Henderson (reinforced concrete structures), tenable at the Imperial College of Science and Technology; A. J. Buller (physiology), tenable at King's College).

The Night Sky in July

Full moon occurs on July 8d. 19h. 37m. U.T., and new moon on July 23d. 18h. 31m. The following conjunctions with the Moon take place: July 7d. 12h., Jupiter 5° S.; July 8d. 18h., Saturn 4° S.; July 17d. 17h., Mars 3° N.; July 19d. 10h., Aldebaran 0·4° S. Mercury and Venus are too close to the Sun for observation. Mars rises at 0h. 30m., 0h. 00m. and 23h. 25m. at the beginning, middle and end of the month, respectively; its stellar magnitude is +0·9 and its distance from the Earth on July 15 is 137 million miles. Mars is now prominent in the eastern sky before sunrise. Jupiter sets at 3h. 05m., 2h. 05m. and 0h. 55m. on July 1, 15 and 31, respectively. It is near the boundary between Sagittarius and Ophiuchus, its stellar magnitude is −2·1 and its distance from the Earth in the middle of the month is 400 million miles. Saturn is also in Sagittarius, setting shortly after sunrise at the beginning of the month, and at 3h. 30m. and 2h. 20m. on July 15 and 31, respectively. Its stellar magnitude is +0·3, and it is in opposition on July 7, when its distance from the Earth is 840 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: July 3d. 21h. 44·0m., x Vir. (*D*); July 19d. 10h. 20·3m., Aldebaran (*D*); July 19d. 11h. 34·1m., Aldebaran (*R*). *D* and *R* denote disappearance and reappearance, respectively. The Earth is at aphelion on July 2, its distance from the Sun being 94·6 million miles.

Announcements

The Council of the National Institute of Industrial Psychology has awarded the Vernon Prize for 1960 to Dr. R. B. Buzzard of the Industrial Psychology Research Group of the Medical Research Council. Dr. Buzzard has been engaged for ten years in research into attendance and absence from work, and has made an exceptional contribution to knowledge in this difficult field.

The Department of Mathematics and Natural Science of the Bavarian Academy of Science, Munich, in celebration of its two hundredth Jubilee, is offering a prize of 8,000 D.M. for the best essay on "Die Rückkoppelung als Urprinzip der Lebensvorgänge". The competition is open to persons with a doctorate or other recognized qualifications. The essay is to be written in the German language and presented by August 1, 1961. Further information can be obtained from Prof. R. Sauer, Bayerische Akademie der Wissenschaften, München 22, Marstall-Platz 8.

The eleventh International Congress of Entomology is to be held in Vienna during August 17–25. Further information can be obtained from Dr. Max Beier, General Secretary, P.A. Natural History Museum, Vienna 1, Burgring 7.

The annual conference of the Institute of Sewage Purification will be held at Scarborough, during June 21–24. Further information can be obtained from the Secretary of the Institute at Maple Lodge, Maple Cross, Rickmansworth, Herts.

The Photoelectric Spectrometry Group is organizing a meeting on "Spectrofluorimetry" at the Inorganic Chemistry Department, South Parks Road, Oxford, on July 8. Further information can be obtained from K. A. Macdonald, Unicam Instruments, Ltd., Arbury Works, Cambridge.

The Danish Atomic Energy Commission is organizing an international summer course on "Plasma Physics" at its Research Establishment, Risö, during August 1–12. Further information can be obtained from the Danish Atomic Energy Commission, Research Establishment, Risö, Roskilde.

The tenth General Assembly and nineteenth International Congress of the International Geographical Union are to be held in Stockholm during August 6–13. Further information can be obtained from the Assistant Secretary of the Geographical Association, Park Branch Library, Duke Street, Sheffield 2.

A joint meeting of the Challenger Society and representatives from the Marine Laboratories (Development Commissioners' Scheme) has been convened at the Zoological Laboratory, Downing Street, Cambridge, during June 23–24. Further information can be obtained from Dr. R. Bainbridge, Zoological Laboratory, Downing Street, Cambridge.

The third Australasian Conference on "Radiation Biology" is to be held at the University of Sydney during August 15–18. Further information can be obtained from Dr. Peter Ilbery, Department of Preventive Medicine, University of Sydney, N.S.W.

The eighth International Symposium on "Combustion" is to be held at the California Institute of Technology, Pasadena, during August 29–September 2. Further information can be obtained from the Office of Industrial Associates, California Institute of Technology, Pasadena, California.

The twenty-first International Geological Congress will be held in Copenhagen at the Mineralogical-Geological Museum of the University during August 15–25. The Congress is being organized jointly by Denmark, Finland, Iceland, Norway and Sweden. Further information can be obtained from the General Secretary, International Geological Congress, Mineralogisk Museum, Øster Voldgade, Copenhagen K.

The tenth annual meeting of holders of Nobel Prizes will be held in Lindau during July 4–8. Among those present who are to speak are Prof. H. A. Krebs (Oxford), Prof. F. Sanger (Cambridge), Prof. G. Domack (Wuppertal-Elberfeld), Prof. C. Heymans (Ghent), Prof. W. P. Murphy (Brookline), Prof. A. Butenandt (Munich), Prof. R. Kuhn (Heidelberg), Prof. A. I. Virtanen (Helsinki), Prof. H. Dam (Copenhagen), Prof. W. R. Hess (Zurich), Prof. Hans von Euler-Chelpin (Stockholm), Prof. G. von Hevesy (Stockholm) and Dr. P. H. Müller (Basle). Further information can be obtained from the Sekretariat der Tagungen der Nobelpreisträger, Lindau (B), Postfach 11.

MINOR ELEMENTS IN NUTRITION

THE 132nd meeting of the Nutrition Society (58th of the Scottish Group) was held in the Hannah Dairy Research Institute, Kirkhill, Ayr, on April 30. It took the form of a symposium on "Minor Elements in Nutrition".

In his introductory remarks the chairman, Dr. D. P. Cuthbertson (Rowett Research Institute, Aberdeen), observed that during the past thirty years a steadily increasing number of mineral elements had been found as permanent constituents of plant and animal tissues. The biological functions of mineral elements present in relatively large amounts, the so-called 'major elements', were well defined, but the role of many of the minor elements in tissues was still, as yet, unknown. This might arise partly from the technical difficulties associated with estimating trace amounts of such elements; but with the increased use of modern techniques it is expected that some at least of these minor elements with an unknown function will be found to have an essential physiological role in the tissues.

For the purposes of this symposium, a minor element was defined as a metal generally belonging to the transitional group of the Periodic Table (iodine, fluorine and selenium being exceptions) which is present in the organism in concentrations of not more than 1 in 20,000, or 50 p.p.m., and serves therein an essential function as an oxygen carrier or in some enzyme system, hormone or vitamin.

The first paper, on "Trace Elements in Scottish Soils", was given by Dr. R. L. Mitchell (Macaulay Institute for Soil Research, Aberdeen). Dr. Mitchell pointed out that the realization of the importance of trace elements in animal nutrition had led to a requirement for maps or other means of ascertaining the probable trace element status of the soils of any locality. In contrast to regions with extensive areas of soils derived from uniform parent material such as the great continental plains, where the trace element status of the soil can often be related directly to pedological factors and can be readily mapped on small-scale maps, conditions in Scotland are much more complex and such generalization is impossible.

Scotland is an area of complex geology in which the parent rocks contain highly variable amounts of the biologically important trace elements, and several periods of glaciation have produced surface drift which further complicates the picture.

Dr. Mitchell then discussed the distribution of trace elements in soils and soil parent materials. Trace elements are located in major rock-forming minerals as a result of substitution for major elements of similar ionic radius in the crystal lattice. Elements of biological importance such as cobalt and copper tend to be concentrated in argillaceous sediments along with molybdenum and selenium. Sandstones and derived sandy soils often have low trace-element contents. The trace-element content of soils can be predicted from the parent material. Unlike the concentrations of major minerals, those of minor minerals can differ widely by a hundred or even a thousand times from soil to soil. A semiquantitative estimate of trace-mineral content or even the nature of the parent material will usually suffice for an assessment of soil status. The availability of trace elements is influenced by rate of weathering of soil minerals and pedological factors affecting the rate of weathering.

Uptake of available minerals by the plant is affected by agricultural practices such as liming, which alters the soil *p*H, and drainage. Under Scottish conditions drainage is the factor most influencing the mobilization of trace elements. With poor drainage there is a larger soil concentration of trace elements, and herbage contents of more than 10 p.p.m. molybdenum have been recorded under such conditions. Certain areas of Aberdeenshire are almost completely infertile owing to poor drainage and the accumulation of excessive quantities of extractable nickel in the soil.

Trace-element uptake by animals on herbage is related not only to soil factors such as those mentioned above but also to seasonal changes in plant composition, variation in the relative amounts of different species in the sward throughout the season, stage of maturity of the crop and climatic factors affecting growth.

In the second paper, Dr. E. C. Owen of the Hannah Dairy Research Institute, Ayr, discussed the "Biochemical Function of Some of the Minor Elements". Dr. Owen confined his remarks to cobalt, zinc, vanadium and manganese. As an essential part of vitamin B_{12}, cobalt is essential for non-ruminants to prevent anæmia and to ensure optimal growth on food of vegetable origin. Vitamin B_{12} can be synthesized by ruminants providing they are supplied with sufficient dietary cobalt. In addition, both ruminants and non-ruminants probably need cobalt in ionic form for the activity of a number of enzymes. After describing typical symptoms of cobalt deficiency in animals, Dr. Owen pointed out that people eating only food of vegetable origin run a special hazard in regard to vitamin B_{12} deficiency in that a high intake of folic acid from greenstuff raises the requirement for vitamin B_{12}. The distribution of vitamin B_{12} in Nature is consistent with a general belief that only bacteria can synthesize it from cobalt salts.

Zinc is a constituent of the carbonic anhydrase of bovine erythrocytes and certain organs of the body and of plant tissues. Carbonic anhydrase is inhibited by sulphonamide, but apparently not through the zinc of its molecule. Studies on the skin disease of pigs called parakeratosis show that high dietary calcium aggravates the condition probably by causing a more rapid turnover of zinc and thus raising the requirement. It is claimed that the effects of excess calcium can be nullified by adding citric or acetic acids to the ration.

It is of interest that pigs suffering from parakeratosis show more variable alkaline phosphatase activity than control pigs and that zinc has been suggested as a specific activator of alkaline phosphatase. Other enzyme systems associated with zinc are alcohol dehydrogenase of yeast and equine liver, glutamic dehydrogenase of bovine liver and lactic dehydrogenase of rabbit skeletal muscle. Dr. Owen thought that the turnover of zinc may be of more importance than the actual concentration in tissues.

In the third paper, Dr. C. F. Mills (Rowett Research Institute, Aberdeen) described "Comparative Studies of Copper, Molybdenum and Sulphur Metabolism in the Ruminant and the Rat". Dr. Mills pointed out that elevated levels of molybdenum and sulphate in the diet of the sheep gave rise to depletion of copper stores and the appearance of symptoms of copper deficiency. The mechanism of action of molybdenum and sulphate under these conditions is unknown. Studies with the rat have shown that high dietary molybdenum contents lead to reduced growth-rate, failure of hæmoglobin synthesis, and production of skeletal abnormalities. That this indicated an interference with normal copper metabolism was shown by increasing dietary copper levels and thus preventing the deleterious effects. It has further been shown that high dietary levels of molybdenum cause accumulation of copper in the liver of the rat. This is in marked contrast to the experience with sheep, where a *depletion* of tissue copper reserves usually occurs.

Excess dietary sulphate also has a contrasting effect when fed in high-molybdenum/low-copper diets. In sheep, increase in dietary sulphate precipitates a rapid decline in liver copper levels, but in the rat it prevents the abnormal accumulation of copper in the liver and improves the growth-rate, hæmoglobin development and skeletal development. In both rat and sheep, adding sulphate to the diet led to an increased output of urinary molybdenum and a fall in tissue molybdenum levels.

These marked species differences led to an investigation of the fate of sulphate in the digestive tract. In the ruminant, sulphide is formed rapidly by micro-organisms, and experiments on feeding molybdenum, sulphide and copper to rats in semi-synthetic diets were carried out. It was found that the sulphide oxidizing system of the rat liver was markedly depressed by feeding molybdate. This suggested that the detoxication of sulphide may be restricted in tissues high in molybdenum.

Among other enzyme systems found to be influenced by high dietary levels of molybdenum are alkaline phosphatase and liver cytochrome oxidase. In connexion with the latter, it is interesting to note that liver cytochrome oxidase activity may be drastically reduced in the copper-deficient rat.

Comparable work is in progress feeding sheep on diets enriched with molybdenum and sulphate. It would appear that the presence of molybdenum increases the rate of reduction of sulphate to sulphide in the rumen. The presence of molybdenum and sulphate depressed the content of soluble copper in the rumen and abomasum. These results might indicate the formation of insoluble copper sulphide, a poor source of copper for sheep.

However, the true significance of the increased sulphide levels in the rumen of sheep following ingestion of molybdenum, in relation to changes in soluble copper concentration, are not yet clear, and in current work to study this aspect the possibility of more extensive effects of molybdenum on sulphur metabolism in the digestive tract is being considered.

In his paper on "Selenium in Animal Health", Mr. G. A. M. Sharman (Veterinary Investigation Service, North of Scotland College of Agriculture, Aberdeen) observed that selenium was a relatively scarce element which was hard to estimate chemically. The lowest soil concentrations are expected in light soils derived from Old Red Sandstone, carboniferous sandstone rocks or certain granites. Concentrations of 0·05 p.p.m. or less might be expected in such soils and might well give rise to deficiency states in grazing animals. Relatively higher values are found in sedimentary rocks, certain shales having values as large as 0·25–1·2 p.p.m. There are certain areas in western Canada and the United States, Mexico, Colombia, Ireland, Israel and Australia where soils containing even higher contents of selenium are to be found, and because the vegetation is high in selenium there is a problem of toxicity among grazing animals. On such seleniferous soils plants absorb very little or a great deal of selenium from the soil depending on the species. Some plant species contain as much as a thousand times more selenium on the dry basis than the soil on which it was grown.

Selenium is the only element known to be absorbed by plants in sufficient amount to cause death from acute poisoning when eaten. For farm animals, dietary concentrations above 10 p.p.m. would appear to be lethal and for chicks a level of 1 p.p.m. produced definite changes in blood constituents. Ingestion of sub-lethal amounts of selenium for long periods leads to its accumulation in hair and hide. Chemical estimation in hair is therefore a guide to selenium status. Cattle from seleniferous areas had hair with selenium content ranging from 10 to 25 p.p.m. compared with the normal 1–4 p.p.m.

It has been shown recently that in rats, chicks and certain farm animals, symptoms of vitamin E deficiency may be alleviated by feeding traces of selenium, and in some cases selenium appears to be essential. New York workers have shown that rations containing 1·1 p.p.m. selenium fed to pregnant ewes prevented white muscle disease in lambs, whereas rations containing 0·01–0·08 p.p.m. did not. Similar experiences have been reported from elsewhere.

Enzootic muscular dystrophy in calves in the north of Scotland was cured by daily dosing with 0·25 mgm. selenium as selenate, or by weekly dosing with 200 mgm. α-tocopherol.

Mr. Sharman emphasized that selenium should not be added to commercial mineral mixtures for farm animals because of the small margin of safety between essential and toxic levels.

In the final paper, on "Adventitious Metals in Processed Foods", Miss E. M. Chatt (British Food Industries Research Association, Leatherhead, Surrey) directed attention to the rapid advances in food production, processing and technology which had taken place this century. She pointed out that increased production of flesh foods led to increased production of crops, giving rise to increased hazard from pests, which had to be controlled by chemical sprays or dusting. Some of these methods of chemical control give rise to contamination with toxic metals which can persist and be carried over into the finished product. Adventitious metals may be present therefore in many raw materials before they arrive at a factory for processing, and in the factory further contamination is liable to occur. In the case of canned foods, contamination also occurs by absorption of tin and iron during storage.

In drawing up recommendations for limits of traces of metals in foods the Food Standards Committee of the Ministry of Agriculture, Fisheries and Food is guided by what is judged to be good commercial practice. All paint in factories should be lead-free and of the rust-resisting variety. Condensation

should be avoided by suitable factory design. Equipment should preferably be made from metals not readily subject to abrasion, such as stainless steel. Miss Chatt gave many examples of ways of avoiding contamination, including methods of inspection and the manufacture and lacquering of cans, and also gave a comprehensive list of the commoner metallic contaminants sometimes encountered in foods—arsenic, lead, copper, zinc, iron, nickel, mercury and antimony. J. DAVIDSON

NITROGEN IN TROPICAL AGRICULTURE

A SYMPOSIUM on nitrogen in tropical agriculture was held in Brisbane during February 23–24. It was organized by the Commonwealth Scientific and Industrial Research Organization at the Cunningham Laboratory, the headquarters of the Division of Tropical Pastures. Although the majority of the papers were by officers of the Organization, several other organizations concerned with research in agricultural and pastoral industries in tropical Australia also contributed.

In his foreword to the conference proceedings, Dr. J. G. Davies (chief of the Division of Tropical Pastures) described the objective of the symposium as "to review existing information on the role of nitrogen in the agriculture of the tropics and subtropics, with emphasis on those aspects which are relevant to the problems of northern Australia and Queensland in particular".

The symposium was highly successful on two counts: it brought together under ready reference a number of reviews on topics of the utmost importance to the future development of tropical agriculture, and equally significant, the papers and discussions demonstrated many serious gaps in information on the role of nitrogen in the tropics and showed where further research is required.

Four papers in Session 1 (general problems of nitrogen economy) dealt with the processes by which nitrogen is gained and lost by the soil/plant system and with the nitrogen economics of natural plant communities and the grazing ruminant animal. D. O. Norris and E. F. Henzell, who considered the processes by which nitrogen is added to the soil/plant system, emphasized the widespread nature and importance of nitrogen deficiency in limiting pasture and crop production in the tropics and concluded that, despite the difficulty of estimating the efficiency of fixation, there was evidence that tropical legumes, under favourable conditions, were equal if not superior to temperate legumes as nitrogen fixers. A. E. Martin and G. W. Skyring dealt with losses of nitrogen from the soil/plant system, listing leaching, crops, erosion, fire and volatilization as factors involved. They considered that some of the losses due to leaching, and most of the losses of gaseous nitrogen reported in the literature, were exaggerated, and suggested that the standard analytical techniques were likely to give lower gains and higher losses of nitrogen than were, in fact, experienced. J. E. Coaldrake emphasized the much slower turnover of nitrogen in natural communities compared with pastures, and joined with Norris and Henzell in pointing out that important accretions of nitrogen came from non-legumes. In reviewing the nitrogen intake and excretion of grazing animals, I. W. McDonald discussed the varied requirements of different classes of ruminant animal, stressing that they retained, on average, only 15 per cent of ingested nitrogen. Discussion topics included the possible importance of free-living nitrogen-fixing organisms in the nitrogen economy of the tropics and the need to ascertain the nitrogen requirements of ruminant animals in tropical environments.

Session 2 (soil nitrogen) consisted of three papers on nitrogen status and nitrogen transformations in soil. G. D. Hubble and A. E. Martin described the nitrogen status of Queensland soils and noted similar modal nitrogen contents in soils from northern and southern Australia (both of which were less than for East African soils); it was apparent that the total nitrogen of surface soils in Queensland was more highly correlated with total phosphate than with annual rainfall. G. W. Skyring and Barbara J. Carey described the microbial species involved in nitrification and denitrification, their physiology and biochemistry and techniques of isolating and culturing them. Although denitrification occurred in apparently well-aerated soil, no denitrification was detectable in cultures containing measurable quantities of oxygen. Nitrogen transformations in soil were considered by A. E. Martin, who referred to recent tracer work with nitrogen-15 in the United States and Sweden which had shown that mineralization and immobilization of nitrogen were simultaneous processes; only net effects had hitherto been measured. He noted that, while ammonia was important, nitrate was not necessary to the microbial cycle. Important topics in discussion were the factors promoting and inhibiting nitrate formation, the carbon/nitrogen ratio and its indication of soil nitrogen availability, and methods of determining the availability of soil nitrogen. Instances were quoted where, although 80 per cent of the nitrogen present in the original rain forest vegetation remained in the soil, nitrogen shortage was still severely limiting pasture growth.

Session 3 (nitrogen fixation) contained two papers. D. O. Norris, in his review of the biology of nitrogen fixation, showed that the classical temperate legume/rhizobium symbiosis was atypical of the Leguminosae as a whole, which were essentially of tropical origin; he also emphasized the wide range of plants and bacteria involved in fixation. C. S. Andrew considered the growth of legumes in acid soils and used this criterion as a measure of symbiotic efficiency. The use of plants other than conventional herbaceous legumes, for example, blue-green algae or nodulating non-legumes, to build up nitrogen in the system was discussed. The mechanism by which phosphate stimulated nodulation also received attention.

Two papers in Session 4 (plant nitrogen) dealt with the pathways and processes of nitrogen metabolism in plants. Protein synthesis was the main theme of M. P. Hegarty's paper on nitrogen uptake and transformations. He reviewed the evidence pointing to ammonia as a key intermediate and described the primary synthesis of amino-acids and their subsequent elaboration. Interference with amino-acid metabolism caused by mineral deficiency was

described by R. G. Coleman. He showed that mineral deficiency was frequently associated with disruption of enzyme activity; amino-acids may accumulate, and symptoms of deficiency result. Discussion emphasized the complexity of mineral imbalance which could affect the level of other nitrogen compounds, for example, purines and pyrimidines, as well as amino-acids. The comparative insensitivity of ruminant animals to changes in the proportions of nitrogen compounds in their diet was attributed to the profound modifications brought about by microbial metabolism in the rumen.

Session 5 (nitrogen for crops) contained five papers. I. F. Phipps described the manufacture and use of nitrogenous fertilizers, dealing chiefly with Australia, where about 125,000 tons were used annually, mostly in the form of ammonium sulphate, although the popularity of urea was increasing. The papers on nitrogen fertilizing of crops were by L. G. Vallance (sugar), F. Chippendale (tobacco and horticultural crops), W. J. Cartmill (cotton) and S. A. Waring (grain and forage crops). One hundredweight of ammonium sulphate increased the yield of sugar by 25 hundredweight in Australia: the highest commercial application was 5 hundredweight ammonium sulphate per acre. There was considerable disagreement about the effects of different sources of nitrogen on tobacco quality, although the nitrate form was favoured in Queensland. With cotton in Queensland, responses to nitrogen were related to moisture availability; best results had been achieved where nitrogen fertilizer and irrigation were combined. Among possible adverse effects from nitrogen fertilization of grain crops were lodging, excessive nitrogen content in malting barley, and actual yield depression with droughted sorghum. Delegates spoke of the different soil depths exploited by different forage crops, the superiority of slow-acting nitrogenous fertilizers for some crops, and the growing popularity of urea as a source of nitrogen.

Session 6 (nitrogen for pastures) comprised two paper on sources of nitrogen, both including consideration of the influence of the grazing animal. W. W. Bryan considered the three symbioses legume/rhizobium, legume/grass and pasture/herbivore. World information on these relationships was almost wholly confined to temperate conditions; because of the many important differences of the tropical environment, a thorough study of these symbioses in the tropics was needed. Discussion revealed conflicting points of view on the importance and duration of the effects of nitrogen voided by the grazing animal. The need for perennial legumes capable of exploiting an improved soil environment, further information on native tropical pasture legumes as nitrogen donors and fundamental data on the mechanism of legume adaptation to difficult environments was emphasized. In reviewing the use of nitrogen fertilizer on pastures, E. F. Henzell pointed out that the widespread nature of nitrogen deficiency in tropical regions was accentuated by the lack of pasture legumes. There was a number of grass species with a growth potential appreciably greater than temperate grasses when nutrition was adequate. Because of the relatively low returns from pastoral farming and the high cost of nitrogenous fertilizers, insignificant quantities were now used on tropical pastures.

Discussion at the symposium inevitably dwelt on the fact that data applicable to the tropical environment were sadly lacking in such diverse fields as pasture utilization, nitrogen fixation by free-living organisms, soil nitrogen availability and the nitrogen metabolism and requirements of tropical pasture and crop plants. Those participating, most of whom were actively engaged in research on these and related fields, expressed the hope that the symposium would help to focus attention on the important problems of nitrogen in tropical agriculture.

A. G. Eyles

RHEOLOGY IN AUSTRALIA

A SYMPOSIUM on "Visco-Elastic Systems" was held during February 16–18 at the Division of Forest Products, Commonwealth Scientific and Industrial Research Organization, Melbourne. Organized by the host laboratory, the meeting was attended by more than fifty delegates from universities, industrial concerns and research institutes, and it foreshadowed some of the activities of the nascent Australian branch of the British Society of Rheology.

Opening the conference, Dr. F. W. G. White, chairman of the Organization, stressed the need to place industrial activity on the most mature and fundamental scientific basis. He welcomed the move to form a rheological society in Australia, which would provide more opportunity for co-operation on common problems in different scientific fields.

The topics were grouped into six sections. In the first of these, "Structural Interpretation", B. J. Rigby reviewed theories of rate processes, and illustrated the use of the Burte-Halsey theory by its application to the rheological behaviour of wool. R. S. T. Kingston applied rate-process theory to the analysis of time-dependent mechanical behaviour of wood at high stresses. A qualitative change in the flow process was indicated by a stress region where energy constants underwent sudden changes.

H. G. Higgins presented a theoretical account of the behaviour of hydrogen-bonded materials, based on the work of Prof. A. H. Nissan. Chemical substitution of hydroxyl groups in cellulose can confirm the basic assumption that the mechanical response is controlled by the number of hydrogen bonds per unit volume. Dr. N. W. Tschoegl gave an account of his studies on monomolecular cereal protein films. The influence of the substrate on the visco-elastic properties of the film yields much information on the nature of bonding in the film. A. W. McKenzie discussed the influence of inter-fibre bonding in a paper on the tensile stress-strain behaviour. A qualitative change in the orientation of fractures with increased bonding is interpreted as an increase in shear strength relative to tensile strength. R. C. Gifkins, after briefly surveying the subject of deformations in crystals, suggested that apparent viscous relaxation in grain boundaries may be an interaction of dislocations and vacancies, that is, a discontinuous crystalline process.

In the second section, "Heterogeneous Systems", Dr. F. A. Blakey discussed the relative contribution of the cement paste and of the aggregate to the creep

of concrete. J. De Yong, in a paper with H. G. Higgins, described draining studies on a pad of cellulose fibres, representing an early stage of paper formation. Compressibility of the porous medium complicates both the Kozeny and the drag equations. L. Dintenfass presented a rheological classification of suspensions, separating the properties of the solid and the liquid phases. He correlated colloidal structural properties such as sedimentation volume with the flow-curves of paints and other systems.

In the following section, "Hygroplasticity", several papers were devoted to wood and one to wool. Dr. G. N. Christensen described the effect of rheological properties on sorption. He had observed that the rate of water sorption by thin layers of wood is inconsistent with diffusion control, and suggested it might be dependent on the relaxation of swelling stresses. Two important practical effects arising on drying wood, namely, 'collapse' and 'set', formed the subject of a paper by Dr. W. G. Kauman, who correlated macroscopic observations with the molecular processes in the cell wall. M. Feughelman discussed the effect of humidity on stress relaxation in wool, and suggested a model in which hydrogen bonds break in the presence of 'mobile' water. L. D. Armstrong presented experimental evidence on the great increase in creep of wood when changes in moisture content take place while the material is loaded. The influence of changes in moisture content was also observed by J. W. Gottstein, who used strips of wood veneer held at constant deformation and subjected to humidity cycles.

The fourth section was concerned with "Specific Materials". Dr. F. A. Blakey reviewed factors influencing the creep of gypsum plaster, and again emphasized the effect of free moisture content on the stress-strain-time relations. Various practical requirements of glazing compounds were discussed by E. R. Ballantyne, who compared the properties of oil-based and rubber-based compounds. C. Massey described tests on several thermoplastics with reference to their suitability for model structural analysis.

In a section on "Instruments", Dr. B. I. Aldrich, in a joint paper with E. L. Newman, described a Couette viscometer designed for testing syrup during fractionation; it accommodates a wide range of shear stresses and has close temperature control. K. G. Martin described a micro-viscometer and discussed factors in its operation.

"Continuum Theory" was the subject of the last section. Dr. P. U. A. Grossman discussed Prof. K. Weissenberg's ideas leading from an assumed restriction on stress directions for a given strain history to observable phenomena, such as cross-stresses.

A policy session was held, at which the possibility of future co-operation in the field of rheology was discussed. In New South Wales the branch of the Society of Rheology has already held several meetings and has arranged a full programme of activities for the current year, including lecture courses on rheology. Plans were discussed for broadening the interest in this field, and for holding future meetings both at the local and national level. P. U. A. GROSSMAN

GAS CHROMATOGRAPHY

THE Gas Chromatography Discussion Group of the Institute of Petroleum Hydrocarbon Research Group held its annual general meeting in conjunction with an informal symposium at the Brunel College of Technology, Acton, London, W.3, on April 22. The secretary, Mr. D. H. Desty, reported that the Group had had another successful year in which it consolidated its position following the rapid expansion of the past few years. Interest in the Group's activities has been well maintained and its status in the field is now well recognized on an international basis.

The abstracting service organized by the Group has made rapid progress, and more than 100 British and foreign journals are abstracted regularly by a team of 25 abstractors. The inaugural volume of "Gas Chromatography Abstracts", 1958, was published by Butterworths, London, on May 5, and has largely been the work of Mr. C. E. H. Knapman, the editor of the Group's editorial committee. This volume provides abstracts and, more particularly, a detailed subject index for 1,468 papers in this field published up to January 1, 1959. Succeeding volumes will appear annually.

Dr. A. T. James and Dr. D. Ambrose have taken part in the preparation by an international committee of preliminary recommendations on the nomenclature and presentation of gas chromatographic data, which were presented to the Analytical Section of the International Union of Pure and Applied Chemistry in September, 1959. These have now been approved in the International Union for publication. The Group's financial position is very sound, and its financial year has been adjusted to coincide with that of the Institute of Petroleum Hydrocarbon Research Group (that is, to the end of September). No change in the annual fee (1 guinea), which is now primarily intended to cover the cost of publications issued by the Group, is therefore necessary.

The informal symposium which followed the annual general meeting was organized and introduced by Dr. J. A. Skellon of Brunel College of Technology, who commented on the active research interests of the College in the field of gas chromatography. The first paper, and perhaps the highlight of the meeting, was that by Dr. J. H. Purnell on the modification of solid supports. Dr. Purnell described measurements, made in his laboratory, of the adsorptive properties of 'Celite' and brick dust which show that whereas 'Celite' has the lower adsorption, brick dust has the more reproducible properties. These experiments were made with the view of studying treatments of the support which might reduce adsorption and minimize tailing of peaks. One promising method of treatment is the use of hexamethyldisilazane, which reacts with hydroxyl groups. Its practical application is simple and the treated support is unwettable and has reduced adsorption. Although chromatograms obtained on the treated support are improved, a further improvement is obtained if 0·1 per cent polyethylene glycol is added to the column with the stationary phase. One disturbing fact is that treatment of the support in this way has a slight effect on values of the specific retention volume even when the columns contain as much as 20 per cent of stationary phase.

The other contributions to the meeting concerned the study of pyrolysis products. Dr. J. Girling described studies on the identification of the primary products of the pyrolysis of coal, in which field the introduction of gas chromatography has enabled the scale of experiments to be reduced from 20 kgm. to 20 mgm. A 20-mgm. sample is heated to a series of steady temperatures ranging from 50° up to 700° C. and the products of pyrolysis at each temperature are swept into a refrigerated trap. The analytical sample is injected on to the column, containing polyethyleneglycol or squalane, by means of an automatic valve of 0·1 ml. capacity; there was considerable interest in the design of this valve, the fixed sample volume of which is formed by a length of 1-mm. bore nylon tubing between two sections of the tubing which are compressed by cam-operated plungers. Dr. J. Blenkin described the analysis of hydrocarbon elastomers by combined pyrolysis and gas chromatography. It was hoped that elastomers could be identified if the monomers from which they are made could be obtained from them and analysed by gas chromatography: to a large extent this hope has been realized. Samples are pyrolysed by dropping them into a heated silica tube attached to the apparatus, so that the products of pyrolysis are swept into the column and chromatographed; for example, polybutadiene gives a chromatogram of butadiene, natural rubber one of a mixture of isoprene and dipentene. Dr. Lehrle described similar experiments in which the degradation was carried out in stages. A sample is placed in a coil of resistance wire in the form of a basket which is located at the entrance to the column. The temperature of the wire is varied by altering the current passing through it, and within half an hour a series of chromatograms can be obtained corresponding to the degradation of the one sample at each temperature. The series of chromatograms is characteristic and suitable for qualitative and quantitative analysis.

The symposium concluded with a general discussion on sample injection, which was introduced by Dr. Purnell. Most speakers were agreed on the difficulty, if not present impossibility, of injecting representative samples for quantitative analysis which are small enough to be satisfactory with ionization detectors. The lower limit of sample introduction possible with pipettes and syringes is probably about 25 micrograms. Various suggestions—for designs of valves or introduction of the sample in solution—were made, but the simplest proposal for quantitative analysis was made by Dr. A. B. Littlewood, namely, to use a large sample and correspondingly insensitive detector.

D. Ambrose

MEDICAL RESEARCH IN MALAYA

THE valuable research work being done in the vast field of tropical medicine is well brought out in the annual report of the Institute for Medical Research, Federation of Malaya, for 1958.

The Institute is divided into a number of divisions, each of which is carrying out investigations into diseases which are baffling and wide-spread. In the Pathology Division, for example, a curious type of allergic granuloma, apparently hitherto undescribed and believed to be the result of an auto-immunity reaction, has been investigated for some years. The variations in the behaviour of melanocytes when surrounded by atypical or malignant epidermal cells have also been studied.

A number of important investigations are being made in the Division of Nutrition and Biochemistry. Severe anæmia is one of the main complications of pregnancy in Asian women, and one in eight of the beds available for ante-natal patients in the General Hospital, Kuala Lumpur, is occupied by a patient requiring treatment for anæmia. Premature births, deaths of infants and of mothers were all increased in this group of women.

The relationship of hookworm infection to anæmia was also studied: the greater the degree of anæmia, the higher was the proportion of patients with hookworm infection. The use of radioactive chromium enabled calculations of the daily loss of blood apparently due to hookworms; with heavy infections this could amount to as much as 80 ml. (almost 3 fl. oz.) of blood a day. Other studies were made on the changes in blood volume in chronic anæmia, on the factors influencing response to treatment, and on the reasons for the frequent low levels of vitamin B_{12} to be found in patients with megaloblastic anæmia.

Dietary inquiries made in the homes of a number of the patients indicated that various causes, besides poverty, were responsible for the poor diets which finally resulted in their admission to hospital with nutritional anæmia.

In almost every area where sufficient numbers of 'toddlers', children aged 1–4 years, have been examined, examples of protein malnutrition can be found; this is true even in the heart of Kuala Lumpur. Only a few come to hospital; they must be sought out by home visits and detailed inquiries. As might be expected, the condition is most common in isolated areas where communications are poor and unemployment is prevalent. A detailed investigation was made of kampongs on the Perak River where protein malnutrition is common; the diets there were found to be most unsatisfactory, and well below the recommended allowances.

In the Bacteriology and Serology Division, research activities were directed to the production of vaccines to combat epidemics of cholera and smallpox.

The search for Malayan streptomyces which may produce antibiotics continued, but without success; resistance of bacteria to the commonly used antibiotics is becoming more noticeable.

Resistance to the tubercle bacillus is now reaching the point where it presents a serious problem. Judging by sensitivity tests, about 10 per cent of local strains are completely resistant to streptomycin, about 10 per cent are resistant to *p*-aminosalicylate, and 2 per cent to isoniazid. Partial resistance to one or other of these drugs is found in 30–50 per cent of strains tested.

The Virus Research and Medical Zoology Division continued its work on arthropod-borne and other viruses. Further work was done on the survey of animal reservoirs of leptospirosis, and infected rats were obtained from Maxwell Hill in Perak, from Langkawi Island, and from parts of Trengganu. By

contrast, no infections were found in much larger numbers of rats from Selangor coastal region. A new development, still being investigated, was the finding of evidence of frequent infection in one but not another species of water-snake in Kuala Lumpur streams.

Leptospirosis in humans is now recognized as being much more common than had been suspected a few years ago, and the virus laboratory is at present trying to assess the diagnostic value of a relatively simple test, suitable for use in a routine laboratory.

At the request of the World Health Organization, the Entomology Division carried out tests to assess the merits of two types of apparatus in which mosquitoes are held for short periods against surfaces treated with insecticide, and the kill later recorded. The opportunity was taken to include the organophosphorus compound, malathion, in these tests: the duration of effectiveness proved to be about three months, which was longer than had been expected. The importance of this finding is that malathion has had to be used to control bed bugs which have developed resistance to the usual residual insecticides, especially dieldrin. Reports of such resistance were received from three rubber estates and a small mental hospital, and malathion is reported to have given satisfactory results.

An incidental result of house-spraying with dieldrin has been the virtual elimination of head-lice in the children living in the sprayed houses. Whereas in unsprayed areas infestation-rates in both boys and girls were 70–90 per cent, no active infestation could be found in the children where spraying had been carried out for one year.

Other work done at the Institute was by the U.S. Army Medical Research Unit, Malaya. The unit investigated two outbreaks of scrub typhus in the Commonwealth Forces in some detail. One in central Perak, near Ipoh, was unusual in that the disease was mild, the Weil–Felix reactions were negative, and cases occurred in forest during the dry season instead of in scrub terrain in the wet season. *Rickettsiae*, however, were isolated in mice inoculated with blood from several patients, and they appear to belong to the scrub typhus group. Cases from the other outbreak in south-east Johore were typical in nature, and Weil–Felix tests were positive.

The report may be obtained from the Director of Medical Research, Federation of Malaya, Kuala Lumpur.

SURFACE TEMPERATURE OF SWEDISH LAKES AND THE ORIGIN OF RAINFALL

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The Dry Summer of 1959

THE accompanying diagrams (Figs. 1–4) give the distribution of rainfall over Sweden as a percentage of the normal in 1959 (published by the Meteorological and Hydrological Institute of Stockholm). They show very clearly that in the summer of 1959 Sweden shared the scarcity of rainfall characterizing the weather over most of north-western Europe, although it varied from place to place. As will be seen, the whole inland area, generally speaking, with its half-dried-out rivers and reduced crops, is framed in by belts of moisture along the coasts and the Norwegian and the Finnish borders, broadening to their maximum in the north.

The period of deficit in rainfall over the major part of Sweden lasted for the five months May–September, according to the monthly surveys of the Meteorological and Hydrological Institute in Stockholm, from which the following data are cited:

May. Total precipitation averaged 87 per cent of normal, with sub-variations of 300 per cent in the north down to 10 per cent in the south.

June. Total precipitation averaged 61 per cent of normal with sub-variations of surplus in the north and deficit in the south with its minimum in the middle south-west.

July. Precipitation in general 72 per cent of normal with a minimum of 10–25 per cent in the northern Baltic coast-land.

August. Precipitation in general 41 per cent of normal; 100 per cent only in limited areas in farthest north and south.

September. Precipitation as total average 53 per cent of normal except along the northern part of the Norwegian border, and in a belt running east–west from south of Lake Vänern to the coast south of Stockholm with a rainfall 100–200 per cent of normal.

Note. In the following, temperatures are in degrees centigrade, distances and depths are in kilometres and metres respectively; surface temperatures were measured 0·5–1·0 m. beneath the surface.

Rainfall South of lat. 59° 30′ N. and Deviations in Temperature from the Average during July–September 1959

In this part of Sweden the landscape is dominated by the two big lakes, Lake Vänern with an area of 5,500 km.², and Lake Vettern with an area of 1,900 km.².

July (Fig. 1). The average temperature was 2°–2·5° above normal; precipitation 100 per cent and more only in the south inside a triangular area with its base along the Öresund and farther east to the south point of the island of Öland, reaching up to the south end of Lake Vettern; this lake, together with the north part of Lake Vänern falling inside the contour for 50–75 per cent of rainfall, the south part of Lake Vänern again being inside a region of almost total dryness.

August (Fig. 2). Average temperature was 2°–3° above normal. Precipitation was 10–50 per cent of the average. It was up to normal only in isolated areas, one of them around the south end of Lake Vettern whereas Lake Vänern registered 25 per cent and less.

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September (Fig. 3). Average temperature was 0·5°–1·5° above normal. Precipitation was only 10–50 per cent of the average except inside a broad belt of 100–200 per cent of normal stretching from a region south of Lake Vänern eastwards, covering Lake Vettern and farther east passing the Baltic to the island of Gotland, its southern border touching the north point of the island of Öland.

Probable Cause for Heterogeneous Distribution of Rainfall

A study of the diagrams for July–September gives the impression that the surface of Lake Vettern, in the midst of an almost dried-out inland area, exercises a sort of attraction on the contours encircling areas of increased rainfall (July) in some cases being more or less included therein (August–September); by contrast, the surface of Lake Vänern, its neighbour to the west, is generally speaking, avoided by the contours, and in some cases came partly inside regions of almost total dryness (July–August). In order to explain this difference we have to look into the hydrographical conditions characterizing the two lakes.

Lake Vänern. The surface of this lake is a quadrilateral figure of about 70 km. × 70 km. apart from a peninsula cutting into it from the north-west. Its bottom topography over the major part of its area is more or less a plane from 20–40 m. beneath the surface, and sloping slightly west- and northwards where in isolated places depths up to some 90 m. have been sounded. The water in this shallow basin in periods of intense insolation during the summer months easily attains a surface temperature of 20° and higher, which has been the case for months in the hot summer of 1959. Measurements then undertaken in the middle of July by the Meteorological and Hydrological Institute showed a surface temperature of 19·3°.

Lake Vettern. This is farther east; its basin was created by a depression in the Earth's crust, forming a groove stretching south-south-west–north-north-east 128 km. in length by some 20 km. in width, up to 30 km. only where the Göta Canal cuts through the

Table 1

Stations	(1)	(2)	(3)	(4)
Date (July)	22	20	20	20
Wind	?	N., faint	N., faint	quiet
Air temp. (°C.)	?	16·3°	15·8°	17·0°
Water temp. (°C.)				
(*a*) Surface	18·2°	17·7°	15·25°	12·8°
(*b*) Soundings				
At 54·5 m.	6·7°	—	—	—
At 15 m.	10·8°	—	—	—
At 5 m.	17·3°	—	—	—
Above depths of	55·5 m.	0·80 m.	0·9 m.	2·8 m.

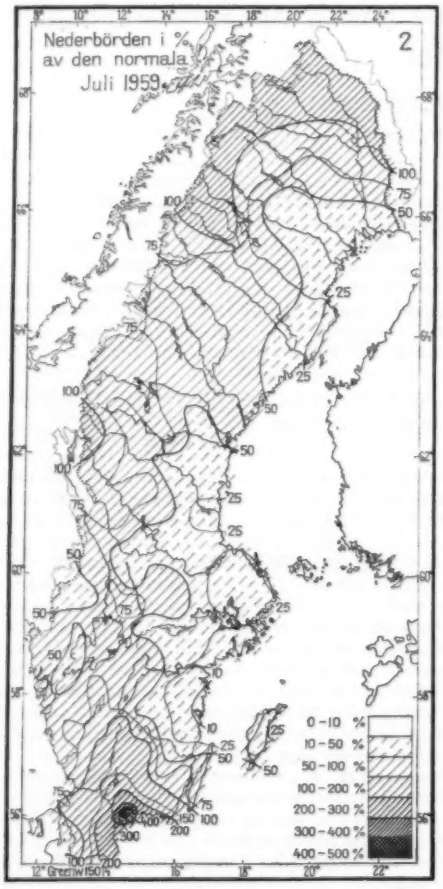

Fig. 1. July

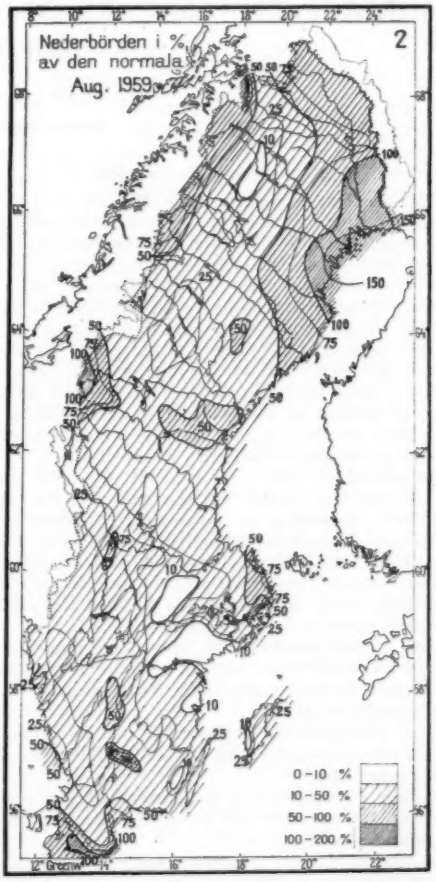

Fig. 2. August

Table 2

1959 Date	July 1	July 11	July 21	August 1	August 11	August 21	September 1	September 11	September 21	November 1	November 11	November 21
Sv. Bj.	10·9°	14·8°	17·0°	18·2°	18·9°	20·3°	11·7°	13·0°	11·3°	8·8°	8·0°	7·1°
Average		14·7°			19·1°			12·0°			7·6°	
Vinga	17·7°	18·4°	19·3°	19·9°	19·1°	18·6°	16·6°	16·5°	15·7°	10·3°	9·9°	7·7°
Average		18·4°			19·3°			16·3°			9·3°	

east border at Motala. Its east and west borders slope down to meet in the bottom of the groove at a depth of some 100 m. running parallel to and some kilometres east of the longitudinal axis of the lake almost from end to end. The water of Lake Vettern is remarkable for its low surface temperature in summer relative to that of Lake Vänern, and also for the perfect clearness of its water, the neighbouring towns taking drinking water by pipe-lines from the Lake. Temperatures were recorded at four points along the borders of Lake Vettern in July 1959 by the Ice Service of the Meteorological and Hydrological Institute. The points of observations were (Table 1); western border: (1) Karlsborg, lat. 53° 30′ N.; (2) Hjo, lat. 53° 15′ N.; southern end of the Lake: (3) Jönköping; eastern border: (4) Gränna, lat. 58° N.

The variations in surface temperature of Lake Vettern in a period of intense insolation giving an average of at least 4° below that of Lake Vänern are

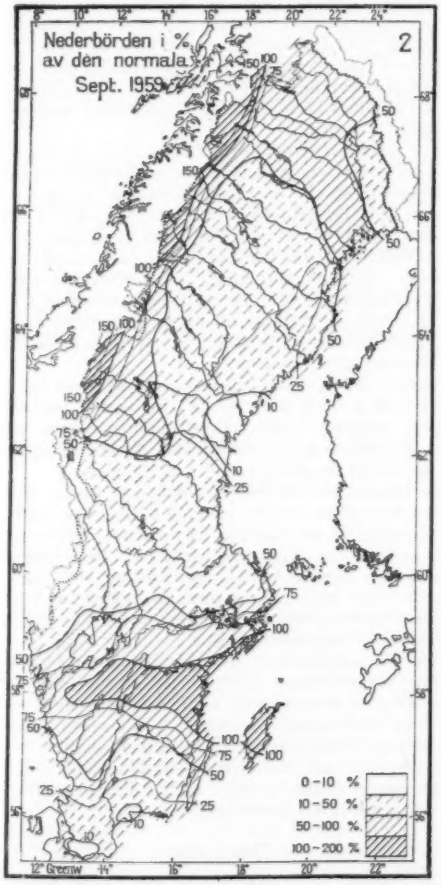

Fig. 3. September

caused by a mixing of the sun-heated surface with colder water from the deeper layers, due to a continuous vertical circulation both ways. The prime agent effecting this is the wind by its tangential pressure on the surface layer, particularly since its direction crosses the Lake perpendicular to its longitudinal axis, namely, east–west and vice versa. The water on the surface thus driven away from the windward border towards the lee border, making colder water from beneath rise and mix with the warmer upper layer, effects a reduction in its temperature. In consequence the surface temperature in Lake Vettern as a rule is higher at the lee and lower at the windward border.

Incidentally, the same phenomenon on a gigantic scale takes place off the west coast of North Africa, where the hot winds from the Sahara always blowing westwards drive the surface layer out from the coast, thereby allowing cold water from the deeper layers to rise to the surface. As a consequence, this part of the North Atlantic has its surface temperature reduced to less than 20° and even to some 16° as far west as the Canaries.

But the tangential pressure on the surface layer, when directed instead more or less parallel to the longitudinal axis of the Lake, namely, north–south or vice versa, drives the water from one end towards the other and makes the water-level rise at the lee and sink at the windward end—which on a reduced scale takes place also in the first case—and gives rise to another phenomenon known as the 'seiches of Lake Vettern'. This phenomenon was studied in the early part of the present century by Dr. F. Bergsten and published by him in a treatise under that title. By bringing the water-level out of balance, the whole bulk of some 80 km.^{3}—some 80 billion tons of water—is set swinging like a gigantic rocking chair on a nodal axis half-way between the ends of the Lake where the movement is nil, increasing to its maximum at either end, the water-level here alternately rising and falling with the same amplitude relative to the horizontal plane. This phenomenon is characteristic especially of oblong lakes and was first studied by Forel in Lake Leman and later by Chrystal and Maclagan–Wedderburn in the Scottish Lochs Earn and Treig. Dr. Bergsten based his work on the methods elaborated by Chrystal and found the seiches of uninodal order in Lake Vettern to have a maximal amplitude of 13 cm. and a frequency, that is, time between oscillations, of 174–180 min.

Variations in barometric pressure have also been found under certain conditions to influence the formation of seiches caused by the winds, for example, when minima pass parallel to its longitudinal axis.

To ascertain the difference in surface temperature between Lake Vettern and the sea areas east and west of southern Sweden swept by the winds before passing over the district of the big lakes, observations from the series registered daily on the Swedish light-vessels *Svenska Björn* in the Baltic east of Stockholm and *Vinga* west of Gothenburg for July–September are given in Table 2. The corresponding observations for November are also given, representing a later

Table 3

1959 November											
Date	2	3	4	5	6	9	12	13	17	18	30
Wind	W. 7	S.W. 10	S.W. 6	N. 3	—	S. 4	S.E. 3	S.E. 8	N.E. 10	S.E. 5	E. 5
		Lee border						Windward border			
$T°$ (4 m.)	9°	9°	9°	8°	7°	7°	7°	7°	7°	6°	6°
Sv. Bj.	8·8°						8°			7·1°	
Vinga	10·3°						9·9°			7·7°	

period of another thermal character to be dealt with later.

The cold surface of Lake Vettern, almost 2,000 km.² in area and situated in the midst of a depression in the highland plateau of southern Sweden, will in hot summers continuously build up a 'mound' of cooled air, making the warmer winds ascend and unload their moisture as shown by the curves of Figs. 1–3 in accordance with the theory of Vilhelm Bjerknes.

The above data from the Baltic and the Skagerak for August 1959 compared with the average values calculated by the Deutsche Seewarte in Hamburg represent a surplus of 3–4 deg., whereas the surface layer of the Baltic in ordinary summers is cooled by the outflow from the rivers in the north transporting water from the melting snow in the mountains of Sweden and Finland. Its total mass, holding a few parts per thousand of salt from a bottom current entering by the Belts from the Kattegat, like a gigantic river is perpetually moving south to its outflow through the Belts and the Öresund into the Kattegat

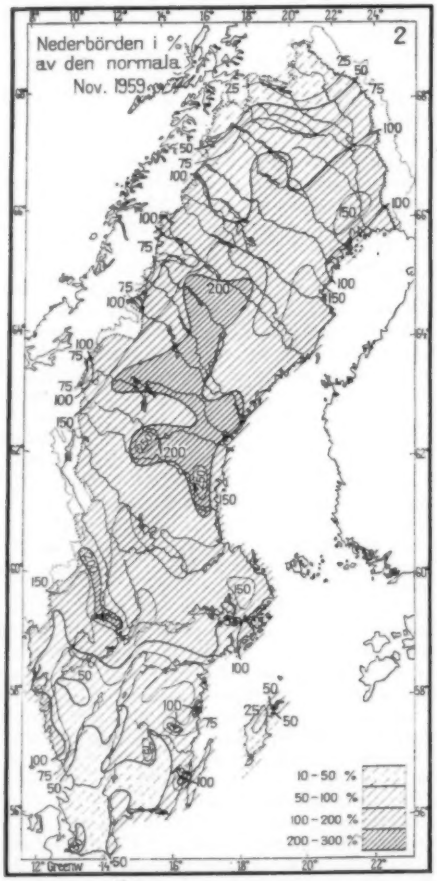

Fig. 4. November

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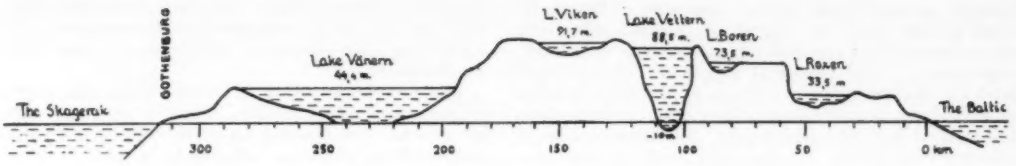

Fig. 5

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MUCOPROTEINS may be defined as conjugated proteins having hexosamine containing oligosaccharides as multiple prosthetic groups. For bovine submaxillary gland mucoprotein, a representative of this class, Hammarsten[1] established in 1888 the presence of a firm chemical linkage between carbohydrate and protein, and he also recognized the strongly acidic character of the compound. In 1901, F. Müller[2] isolated crystalline D-glucosamine hydrochloride from bovine submaxillary gland mucoprotein and demonstrated the presence of acetyl groups in this mucoprotein. During the next fifty years little attention was paid to mucoproteins and scarcely any notice was taken of the important discovery by Blix[3] of a new, though unidentified acidic carbohydrate in bovine submaxillary gland mucoprotein. The situation changed dramatically when Burnet[4] and co-workers showed: (1) that many glandular mucins of human and animal origin, among them the submaxillary gland mucins of ruminants, inhibited hæmagglutination by heat-inactivated influenza virus, (2) that the active principle of the mucin was a mucoprotein and (3) that living influenza virus and an exoenzyme of *Vibrio cholerae* (*RDE*) destroyed this inhibitory capacity.

The elucidation of the chemical reaction underlying the enzymic inactivation of the inhibitory mucoproteins was initiated when Gottschalk[5] and co-

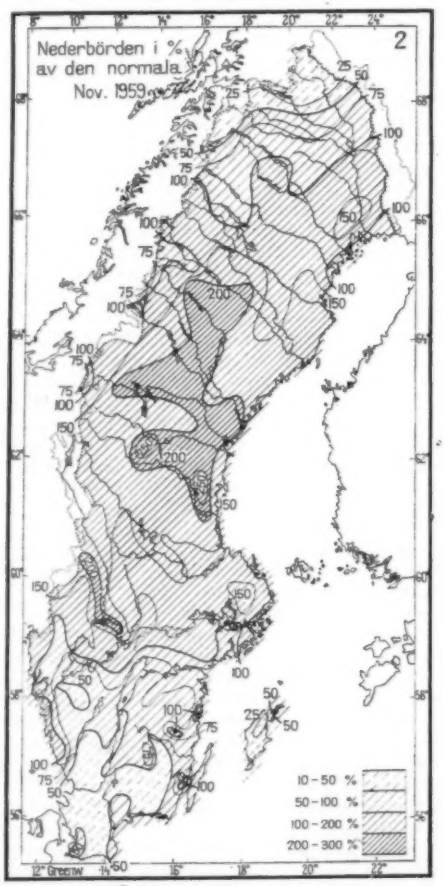
Table 3

1959					November					
Date	2	3	4	5	6	9	12	13	17	18	30
Wind	W. 7	S.W. 10	S.W. 6	N. 3	—	S. 4	S.E. 3	S.E. 8	N.E. 10	S.E. 5	E. 5
		Lee border						Windward border			
$T°$ (4 m.)	9°	9°	9°	8°	7°	7°	7°	7°	7°	6°	6°
Sc. Bj.	8·8°						8°			7·1°	
Vinga	10·3°						9·9°			7·7°	

period of another thermal character to be dealt with later.

The cold surface of Lake Vettern, almost 2,000 km.² in area and situated in the midst of a depression in the highland plateau of southern Sweden, will in hot summers continuously build up a 'mound' of cooled air, making the warmer winds ascend and unload their moisture as shown by the curves of Figs. 1–3 in accordance with the theory of Vilhelm Bjerknes.

The above data from the Baltic and the Skagerak for August 1959 compared with the average values calculated by the Deutsche Seewarte in Hamburg represent a surplus of 3–4 deg., whereas the surface layer of the Baltic in ordinary summers is cooled by the outflow from the rivers in the north transporting water from the melting snow in the mountains of Sweden and Finland. Its total mass, holding a few parts per thousand of salt from a bottom current entering by the Belts from the Kattegat, like a gigantic river is perpetually moving south to its outflow through the Belts and the Öresund into the Kattegat



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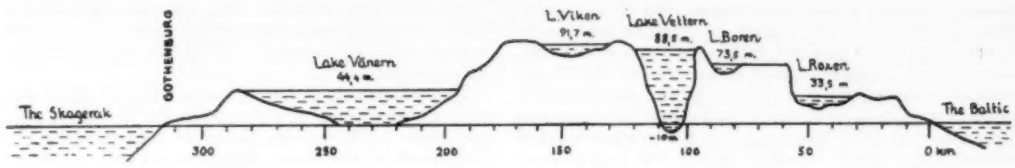

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The elucidation of the chemical reaction underlying the enzymic inactivation of the inhibitory mucoproteins was initiated when Gottschalk[5] and co-

workers isolated a dialysable compound of low molecular weight from the digest of purified inhibitory mucoproteins and purified influenza virus and *RDE*, respectively. The chemical properties of the 'split product' closely resembled those of the acid isolated by Blix[3] from bovine submaxillary gland mucoprotein and termed in 1952 'sialic acid'[4]. By treating bovine submaxillary gland mucoprotein with 5 per cent methanolic hydrochloric acid, Klenk *et al.*[7] obtained the same methoxyneuraminic acid which Klenk had previously prepared from certain brain mucolipids by a similar procedure. Furthermore they isolated from bovine submaxillary gland mucoprotein N-acetylneuraminic acid and on repeating Gottschalk's work identified the crystalline split product as N-acetylneuraminic acid[7].

Concerning the structure of the new sugar acids, Gottschalk[8] proposed in 1955 on the available data a comprehensive scheme with neuraminic acid as the parent structure common to sialic acid, N-acetylneuraminic acid and methoxyneuraminic acid. Neuraminic acid was visualized as an aldolcondensation product of hexosamine and pyruvic acid, N-acetylneuraminic acid as N-acetylated neuraminic acid, sialic acid as N,O-diacetylated neuraminic acid and methoxyneuraminic acid as the methylglycoside of neuraminic acid. By the fusion of pyruvic acid with hexosamine a keto-, deoxy-, hydroxyl- and amino-group become α-, β-, γ- and δ-positioned respectively to the carboxyl group, an alignment favouring cyclization to a pyrrole ring. It was the conversion of the split product to pyrrole-2-carboxylic acid[9] under mildest alkaline conditions which rendered possible the allocation in space of the functional groups, elaborated previously by Blix *et al.*[10] and Klenk *et al.*[7]. The correctness of the scheme was proved by alkaline degradation of N-acetylneuraminic acid to N-acetyl D-glucosamine and pyruvic acid[11] and by the chemical synthesis[12] of N-acetylneuraminic acid from N-acetyl D-glucosamine and oxaloacetate at *p*H 11·0 and 20°. Recently, Comb and Roseman[13] showed that the N-acetylhexosamine fragment of N-acetylneuraminic acid has the steric configuration of N-acetyl D-mannosamine, the ease of the epimerization reaction N-acetyl D-glucosamine ⇌ N-acetyl D-mannosamine accounting for the synthesis of natural N-acetylneuraminic acid from N-acetyl D-glucosamine and oxaloacetate.

The main sugar components of the homogeneous submaxillary gland mucroproteins are N-acetylgalactosamine and sialic acid (in the new nomenclature[14] the generic name of all N- and O- substituted neuraminic acids). Bovine submaxillary mucoprotein contains equimolecular amounts of sialic acid (22·4 per cent expressed as N-acetylneuraminic acid) and N-acetylgalactosamine besides small quantities of other sugars. In ovine submaxillary gland mucoprotein the total carbohydrate (42 per cent) consists practically only of N-acetylneuraminic acid and N-acetylgalactosamine in the molecular ratio 1 : 1.

In the elucidation of the structure of the carbohydrate group of the submaxillary gland mucoproteins the *Vibrio cholerae* enzyme *RDE* proved to be a valuable tool. The liberation of 70 per cent of the total sialic acid, and of sialic acid only, from bovine submaxillary gland mucoprotein by *RDE* suggested a terminal position of the acid; the reducing power of the sialic acid released from the non-reducing bovine submaxillary gland mucoprotein indicated a glycosidic (ketosidic) linkage of the terminal sialic acid to its partner, thus allowing classification of the enzyme as a glycosidase. The further qualification of *RDE* as an α-O-glycosidase was derived from *RDE* action on sialyl-lactose. The enzyme was named 'neuraminidase'[15].

The prosthetic group of ovine submaxillary gland mucoprotein was readily released by mild alkali treatment. On heating an ovine submaxillary gland mucoprotein solution, adjusted to *p*H 11·5 with barium hydroxide, at 80° for 15 min., 22 per cent of the prosthetic groups were released, and this treatment could be repeated several times yielding further quantities of prosthetic groups. The prosthetic group was identified as α-D-N-acetylneuraminyl (2 → 6) N-acetylgalactosamine by elementary analysis, cleavage into its components by neuraminidase, consumption of 4 moles IO_4^-/mole and by conversion to characteristic derivatives[16].

The prosthetic groups are joined through their reducing ends to the free carboxyl groups of glutamic and aspartic acid residues by a glycosidic ester linkage. This was first indicated by an appreciable drop in *p*H concomitant with the release of prosthetic groups when an ovine submaxillary gland mucoprotein solution with an initial *p*H of 10·5 was heated at 80°. Quantitative determination of the number of prosthetic groups released and of the number of carboxyl groups unmasked were in fair agreement. The presence of a glycosidic ester linkage to aspartyl and glutamyl respectively was conclusively proved by the reduction of one of the carboxyl groups of the dicarboxylic acids when ovine submaxillary gland mucoprotein, after pretreatment first with trypsin and then with phenyl-*iso*thiocyanate, was dissolved in anhydrous tetrahydrofuran and acted upon with lithium boron hydride. This reagent is known to effect a reductive cleavage of ester linkages without appreciable action on amide or peptide bonds[17]. The recovery of total dicarboxylic acids of ovine submaxillary gland mucoprotein after such treatment was only 12 per cent of the normal value (10·7 gm. dicarboxylic acids expressed as glutamic acids), indicating esterification of one of the carboxyl groups of the missing 88 per cent dicarboxylic acids. Again the reductive cleavage was accompanied by the release of prosthetic groups. The extent of reductive cleavage of ovine submaxillary gland mucoprotein is limited by its rather low solubility in tetrahydrofuran, even after fragmentation by trypsin and blocking of the N-terminals by phenyl-*iso*thiocyanate to suppress ionization. The true extent of esterification is indicated by the finding that 84 per cent of the prosthetic groups are removed by heating ovine submaxillary gland mucoprotein at *p*H 11·5 and 80° for 4 hr.; this figure cannot be increased by more severe alkaline conditions. About 16 per cent of the prosthetic groups are linked in an alkali-stable O-glycosidic linkage, probably to serine and/or threonine[18].

The quantitative amino-acid analysis revealed a nearly complete complement of amino-acids; however, the content of aromatic and sulphur-containing amino-acids was low and that of glycine, proline and β-hydroxy-amino-acids high[19].

The susceptibility of ovine submaxillary gland mucoprotein to trypsin action is sterically hindered by the prosthetic groups which are bulky substituents at the side chains of frequently occurring amino-acids (Fig. 1). One out of 6·4 amino-acid residues carries a prosthetic group and one out of 16 amino-acid residues is a lysyl or arginyl residue, that is, 2·5 prosthetic groups/trypsin-susceptible bond. Removal by neuraminidase of the terminal

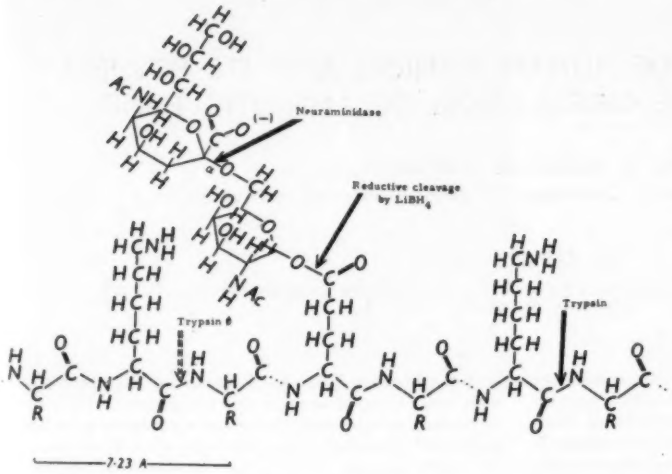

Fig. 1. Diagrammatic segment of ovine submaxillary gland mucoprotein showing: (1) the neuraminidase-susceptible α-ketosidic linkage; (2) the lithium boron hydride-susceptible glycosidic-ester linkage between prosthetic group and the carboxyl of a dicarboxylic acid (here the γ-carboxyl of glutamyl); (3) trypsin-susceptible bonds at the carboxyl of diamino-acids (here lysyl), one accessible to the enzyme, the other sterically hindered by the prosthetic group

N-acetylneuraminic acid units increased by 45 per cent the number of peptide bonds split by trypsin[20].

In the course of these experiments the observation was made that release of N-acetylneuraminic acid by crystalline neuraminidase at *p*H 6·0 decreased markedly the viscosity of ovine submaxillary gland mucoprotein. Quantitative measurements showed that the reduced viscosity of ovine submaxillary gland mucoprotein at *p*H 6·1 decreased from 3·13 to 1·33 on treatment with crystalline neuraminidase (free of proteinases). Since N-acetylneuraminic acid like pyruvic acid is a strong acid ($pKa = 2·6$), this result suggested a dependence of the shape of the molecule on the strongly negative charge of the prosthetic group. To test this suggestion the viscosity of ovine submaxillary gland mucoprotein was measured over a *p*H range of 7·8–1·8 under comparable conditions of ionic strength. There was little change between *p*H 7·8 and 4·3 (isoelectric point of ovine submaxillary gland mucoprotein = 4·7); however, the reduced viscosity dropped from 3·50 to 2·50 between *p*H 4·3 and 1·8, that is, in the *p*H range of decreasing ionization of N-acetylneuraminic acid. This decrease in viscosity was completely reversible on readjustment of the *p*H. These results would indicate that the strongly negative charge of the carboxyl groups of N-acetylneuraminic acid is responsible for the extended shape of the mucoprotein. On enzymic removal of N-acetylneuraminic acid at *p*H 6·0 or on suppression of its ionization the molecule assumes a more compact shape losing thereby its viscous character[21]. Prof. Linus Pauling has suggested to me as a possible structure of ovine submaxillary gland mucoprotein that the protein consists of α-helix segments loosely joined by proline residues. The prosthetic groups would then form a secondary helix around the protein core. It has been shown on models that the average spacing of 6·4 residues offers many possibilities for the formation of hydrogen bonds, in the direction of the helix axis, between adjacent sugar rings. The co-operative effect of the H-bonds and the electrostatic repulsion of the charged carboxyl groups of N-acetylneuraminic acid could preserve a rod-like structure despite the potential flexibility of ovine submaxillary gland mucoprotein at the sites of the proline residues.

In ruminants the submaxillary glands are the main mucus-secreting salivary glands; because of its high mucoprotein content the secretion is very viscous. This secretion together with that of the parotid gland (serous gland) and that of the much smaller sublingual glands (mixed glands) form the saliva. One of the main functions of saliva is to cover the foodstuffs with a lubricant layer of mucus, assisting swallowing and protecting teeth and the lining cells of the mucosa against injury. Of all the substances synthesized by the organism mucoproteins, because of their high viscosity, are especially suited for the functions of lubrication and protection. It seems to be of interest that by such a rather simple expedient as the attachment of strongly negatively charged, multiple disaccharides to the side-chains of the protein core a high degree of viscosity is imparted to the molecule. The wide distribution of neuraminic acid among the viscous epithelial secretions of the respiratory, intestinal and uro-genital tracts may well reflect one, at least, of the physiological functions of this acid. If this concept proves to be true, Nature was wise to incorporate into neuraminic acid the strongest stable organic acid produced in the metabolism of the animal cell.

I am indebted to Dr. G. L. Ada, of the Walter and Eliza Hall Institute, Melbourne, for a generous supply of crystalline neuraminidase.

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ULTRA-STRUCTURE OF HUMAN AMNION AND ITS POSSIBLE RELATION TO THE CIRCULATION OF AMNIOTIC FLUID

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THERE is no doubt that some circulation of amniotic fluid occurs between the human fœtus and mother. According to Plentl[1], from work carried out by the use of deuterium, the exchange is about 450 ml. per hr. at term. Plentl has also demonstrated differential rates of transfer of sodium and potassium ions. The turn-over of sodium in the amniotic fluid is believed to occur at about seven times the rate that potassium is replaced. There is no definite information to show either how such large amounts of fluid are conveyed from the fœtus to the mother, or to account for the passage of various ions at different rates. In an attempt to throw further light on these problems we have been investigating the fine structure of the human amnion at term.

An account of the fine structure of the epithelial layer has been published by Lanzavecchia and Morano[2]. This work was carried out at moderate resolution and relates primarily to changes occurring during development.

Amnion was obtained from patients delivered by Cæsarean section at term. The amniotic membrane was dissected from the chorion, and portions of the placental and peripheral amnion were selected for examination.

Small pieces of the membrane were fixed for 2 hr. in osmium tetroxide solution buffered to *p*H 7·3 (ref. 3). The tissue was then rinsed briefly in distilled water, dehydrated in gradually increasing concentrations of ethanol and finally embedded in a 1 : 3 mixture of *n*-methyl and *n*-butyl methacrylate. Sections were cut with a glass knife and mounted on copper grids previously covered with a thin carbon film. The material was examined in a Siemen's Elmiskop 1*a* generally operated at 60 kV. Micrographs were obtained at initial magnifications varying from × 1,000 to × 40,000. The following is a brief account of the main results so far obtained.

Epithelial Layer. This consisted of a single layer of cells the height of which varied from about 1μ to 25μ. At moderate magnification (Fig. 1) the cells appeared to be covered by a single membrane which, on the surface next to the amniotic fluid, was evaginated to form many short processes or microvilli. Along the lateral aspect, the two membranes of adjacent cells delimited a series of irregularly shaped vacuoles (Fig. 1). Some of these vacuoles simply appeared to extend into adjacent ones; others were connected by fine channels (about 170 Å. internal diameter) formed by the close apposition of the two cell membranes. While the majority of these vacuoles were located in the regions between adjacent cells (that is, were hollowed out in the sides of the cells) a few were seen lying deeper in the cytoplasm. Projecting into some of the vacuoles were a variable number of microvilli, generally less per unit area than seen on the upper surface of the cells. In the basal region the cell membrane was irregular in outline and delimited irregularly shaped vacuoles of various sizes. The appearance of this region, therefore, was similar to that seen along the sides of the cells (Fig. 2). Some micrographs showed that the cell membrane in the basal region was thickened at intervals.

The vacuoles in the lower parts of the cells were generally filled with a substance of moderate density

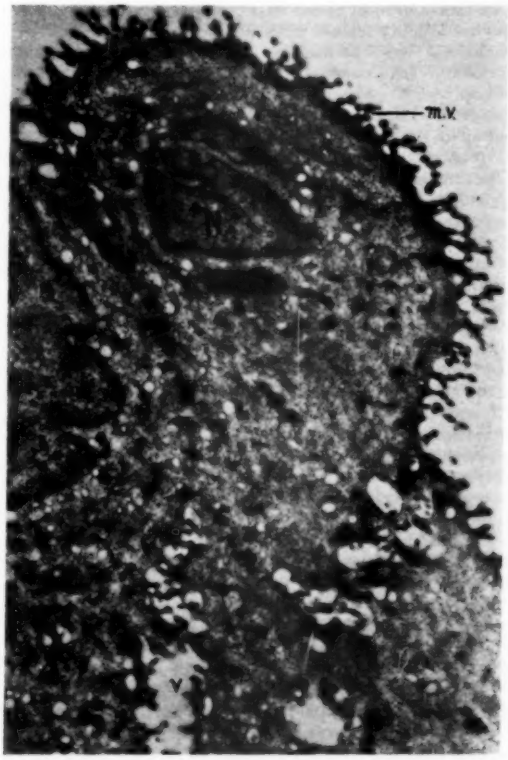

Fig. 1. Micrograph showing the general appearance of the upper part of amniotic epithelial cells. *m.v.*, microvilli; *N*, nucleus; *v*, system of intercellular vacuoles. (×10,000)

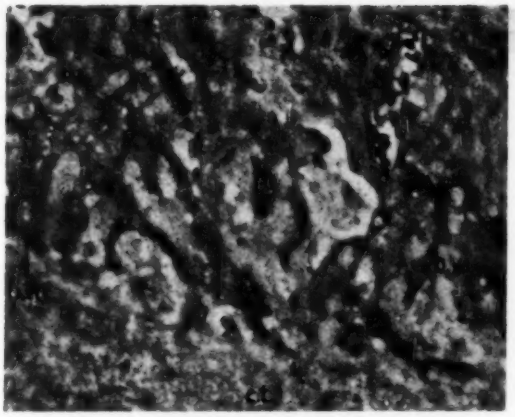

Fig. 2. Micrograph showing the general appearance of part of the basal region of an amniotic epithelial cell. *b.m.*, basement membrane; *b.v.*, basal vacuole; *c.t.*, connective tissue. (×10,000)

which was continuous with, and similar in appearance to, the material forming the basement membrane (Fig. 2). In a few micrographs we observed a similar substance in vacuoles in the upper parts of the cells which communicated with the amniotic fluid. In the majority of our micrographs, however, vacuoles in the upper parts of the cells lacked demonstrable contents.

The cytoplasm of the epithelial cells appeared very dense and granular (Fig. 1). Mitochondria were extremely scarce and when seen were usually in the form of small spheroidal bodies about 0·3μ in diameter. A Golgi apparatus has so far not been observed; if present it was presumably small and inconspicuous. In some cells we observed about two or three (per section) dense bodies each about 1μ in diameter. The nucleus was a large, dense object located close to the upper surface. It was irregular in outline and sometimes fenestrated (Fig. 1).

Various parts of the cells were examined at high magnification and resolution. This revealed that the microvilli were bordered by two closely applied membranes, each about 30 Å. thick. Within the microvilli were many fine rods which, due to the presence of a 'clear' medulla and dense cortex, were canal-like in appearance. The external and internal diameters of these canal-like elements varied; the maximum diameters recorded were 150 Å. (external) and 70 Å. (internal). When seen in cross-section these elements simply appeared to be placed closely together. Rarely, however, elements of similar composition were seen which exhibited the 9 + 2 arrangement found in cilia and flagella[4]. This suggests that occasional cilia existed alongside the microvilli. However, the possibility cannot be ruled out that the 9 + 2 pattern may occur in some microvilli, perhaps during some stage in their development. The canal-like elements penetrated into the cytoplasm to a depth ranging from about 0·25μ to 0·5μ. Due mainly to this there appeared to be a thin layer of particularly dense cytoplasm immediately beneath the microvilli in cells examined at lower magnification (Fig. 1).

When studied at high resolution and magnification, comparatively large areas of the cytoplasm were seen to contain numerous paired parallel lines and circular elements (Fig. 3). These observations are interpreted as showing the presence of membranes arranged mainly in the form of fine canals with some occurring as parallel sheets. The membranes were each about 60 Å. thick, the distance between them (canal lumen) was about 100 Å. Examination of representative parts of the cell surface, where it bordered the lateral and basal system of vacuoles referred to above, revealed many circular apertures (about 75–100 Å. internal diameter) which communicated with the intracellular channels and canals. The evidence suggests, therefore, that amniotic epithelial cells contain an extensive system of canals and channels which communicate directly with the extracellular 'space'. Further details of this system, such as its relationship with the upper surface of the cell and microvilli, are now being studied.

Basement Membrane. This consisted of a thin layer, usually about 0·1μ thick, of a moderately dense substance which penetrated into the vacuolar-like indentations of the cell membrane. At high resolution it was seen to consist of fibrils (each about 200 Å. in diameter) embedded in a material of closely matching density.

Connective Tissue Layer. This relatively deep layer (0·05 mm.–0·5 mm. thick) consisted mainly of fibrils, each about 300 Å. thick and exhibiting faint transverse striations with a periodicity of about 600 Å. The fibrils were branched, the angle of branching usually being about 90°. They were arranged in bundles (not bounded by a membrane) lying in various planes (Fig. 4). Thus in any region a great number of parallel fibrils lay next to a comparable number of similar elements sectioned transversely or obliquely. Such an arrangement accounts for the appearance of a network of fibres in light microscopi-

Fig. 3. Micrograph of the numerous membranes found in various regions of the cytoplasm of amniotic epithelial cells. The membranes are believed to be arranged mainly in the form of canals with some occurring as parallel sheets. Along the cell surface (*c.s.*) are many small circles suggestive of pores. (×100,000)

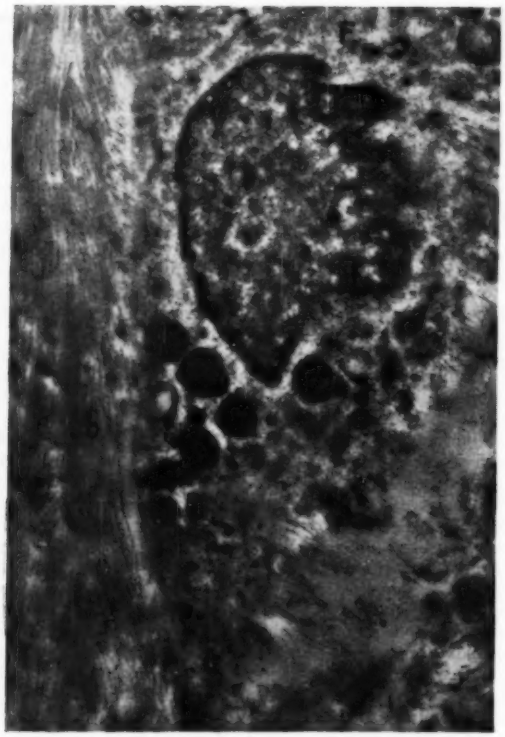

Fig. 4. Micrograph of part of the connective tissue layer showing a fibroblast (*F*) and bundles of fibrils (*b*). Along the lower right-hand side of the cell connective tissue fibrils can be seen partly embedded in cytoplasm. (×9,000)

cal preparations[5]. The morphological evidence, therefore, suggests that the fibrils were reticular fibrils. Typically, in the deeper parts of this layer the fibres had a wavy appearance ; this was probably due to some contraction.

Cells were comparatively scarce and were located in an intermediate zone and in the deeper parts of the layer (fibroblast and spongy layers described by Bourne[5]). These cells were of two kinds : fibroblasts and macrophages. As far as could be determined from individual sections, the fibroblasts in the intermediate zone formed a diffuse network. Parts of the cell surface appeared not to be covered by a membrane. Where this occurred were many connective tissue fibrils projecting from the cell cytoplasm into the extracellular 'space' (Fig. 4). There seemed little doubt that the fibroblasts were actively synthesizing new fibres. The fine structure of these cells varied, either because the cells were of different ages or in different physiological states. Most of them, however, contained an irregularly shaped, dense nucleus (sometimes elongated), a moderately well-developed endoplasmic reticulum (lamelliform and tubular in appearance), mitochondria, Golgi apparatus, groups of fine filaments and several small dense spheroidal bodies. The last-named were possibly glycogen granules[6]. Also present in most cells was a single large mass of material which almost totally occupied the region of the cell in which it was located. This material was bordered by membranes of the endoplasmic reticulum. When examined at high resolution, it was seen to consist of an almost 'clear' matrix within which was a network of greater density. In some cells the network consisted of fibrils (each about 300 Å. in diameter) embedded in a homogeneous ground substance. In other cells the fibrils were scarce or absent and within the ground substance were many small vesicles (each about 500 Å. in diameter). These observations seem to show quite definitely that some connective tissue fibrils were being formed intracellularly.

In the deeper part of the connective tissue layer were elongated cells. The ends of these cells were closely applied and this, together with the fact that the cells followed the wavy course of the connective tissue fibres, made them appear to be articulated. The general appearance of these cells was different from that shown by the cells in the intermediate zone. It seems likely, however, that they were fibroblasts.

The only other cells observed were macrophages. These were very scarce and scattered throughout the connective tissue layer. Within these cells were inclusions which, from evidence obtained by the use of Perl's[7] test for iron (on sections examined under the light microscope), probably contained hæmosiderin.

So far as can be determined on a purely morphological basis, it seems likely that some of the amniotic fluid which passes from the fœtus to the mother, and the accompanying differential rate of turn-over of various ions, may be brought about by the amnion. As a working hypothesis it may be conjectured that the fluid enters the amnion via (*a*) the opening between adjacent cells, where it passes into the lateral system of vacuoles and (*b*) the upper surface, the area of which is greatly increased by the presence of microvilli. Within the cells the fluid presumably enters the complex system of fine canals and channels. The fine canals and channels communicate with the lateral and basal vacuoles and hence with the extracellular space ; this results in an enormous increase in surface area. Within the vacuoles there is presumably some mixing of any solution which may have entered via the two principal pathways. Assuming that some ions travel more readily along one route than the other, then this could offer an explanation (although much over-simplified) of the differential rate of turnover of different ions. Having passed through the epithelium the fluid must permeate the connective tissue layer. Since this layer consists mainly of a network of fibres there is no objection to this on morphological grounds.

Experiments are being carried out to examine the validity of the above hypothesis. This part of our work is at an early stage. However, 30 min. after the injection of colloidal gold in the amniotic fluid a few small particles of gold have been observed in the connective tissue layer, either scattered along the length of some of the fibrils or around small processes of the fibroblasts.

The above work was undertaken during the tenure of a Lawrence Research Scholarship of St. Bartholomew's Hospital held by one of us (G. L. B.).

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LETTERS TO THE EDITORS

GEOPHYSICS

Regular Micropulsations of the Earth's Field at the Equator

THE diurnal variation of occurrence of regular pulsations, *pc*, of the Earth's magnetic field has been studied by many workers and it has been found, in all the cases reported, that the phenomenon is predominantly a day-time one. Typical of the results of magnetic measurements are those obtained in Germany by Angenheister[1], for periods of 30–60 sec., which have a maximum frequency of occurrence at 10–12 hr. L.M.T. The measurements of Earth currents, obtained in the U.S.S.R. by Mme. Troitskaya[2], are representative of that experimental approach and they show a maximum frequency of occurrence around 11–13 hr. L.M.T. Most of the results reported have been confined to mid-latitude stations.

At the symposium on "Rapid Variations of the Earth's Field" held at Utrecht in September 1959, I gave a brief report of an analysis of records of Earth currents obtained at Legon, Ghana (lat. 5° 38′, N., long. 0° 11′ W.). This analysis indicated that, at this station, regular pulsations of Earth currents occur during the night as well as during the day and, in fact, that the maximum frequency of occurrence for the whole day is at approximately 18–20 hr. G.M.T. The diurnal variation of frequency of occurrence of regular pulsations, *pc*, for all days, is shown in Fig. 1*a*, which was the result of an analysis of records of Earth currents obtained between October 1957 and December 1958. More recently, analysis of magnetic records obtained from an induction magnetometer, recording dH/dt at the same station, has shown a similar night-time occurrence of regular pulsations, in addition to the day-time occurrence normally expected. The results of this analysis, which was made for the months of May, June and July 1958, are given in Fig. 1*b*, together with the results obtained from records of Earth currents for the same 3 months. Both graphs show three maxima, occurring at 6 hr., 12 hr. and 20 hr., and in each case the night-time maximum is the greatest. In fact, there is approximately a 50 per cent chance that regular pulsations will occur between 19 and 21 hr. It is probable that the difference in level of frequencies of occurrence in the two cases is due partly to the different sensitivities of the telluric and magnetic recording systems, and partly to the fact that the Earth-current records and magnetic records were examined by the same observer at different times. A separate study has also been made of the degree of subjectivity in the examination of records for the occurrence of these regular pulsations, by having three observers independently analyse the same records. In all cases, the results gave rise to graphs of very similar form to that in Fig. 1*a*, but the mean value of frequency of occurrence differed from one observer to another, a factor which

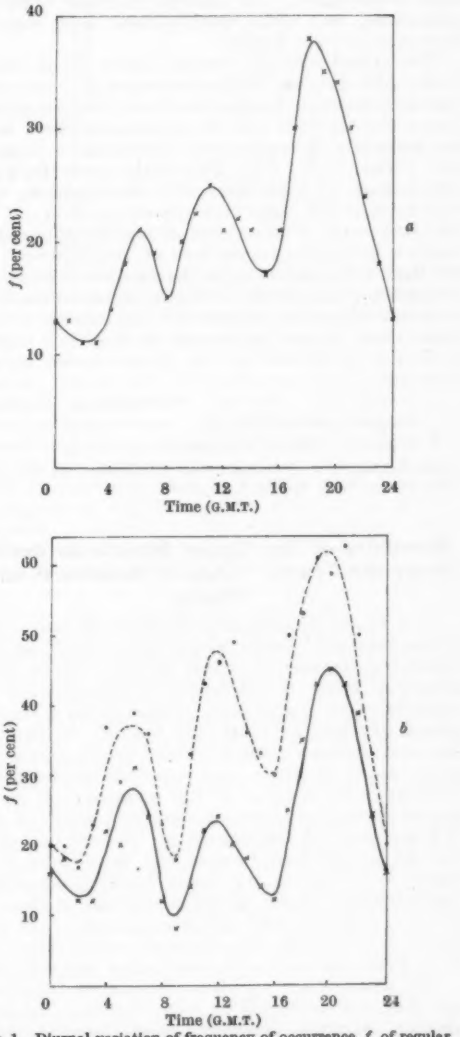

Fig. 1. Diurnal variation of frequency of occurrence, *f*, of regular pulsations at Legon. (*a*) Telluric pulsations for October 1957–December 1958; (*b*) telluric and magnetic pulsations for May–July 1958. Telluric, ———; magnetic, – – – –

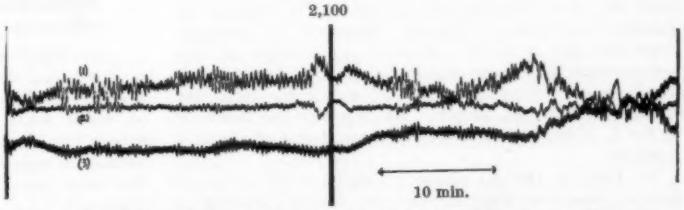

Fig. 2. Example of night pulsations at Legon on both telluric and magnetic traces: May 27, 1958, 2030–2130 hr. (1) E.–W. telluric current; (2) dH/dt; (3) N.–S. telluric current

was not of primary importance in the present study.

This analysis of both telluric and magnetic records and the independent examination of records by three observers confirms the existence of regular pulsations, *pc*, during night-time as well as during the day, in the equatorial region. An example of these night-time pulsations, on both Earth-current and magnetic traces, is given in Fig. 2.

The Earth-current results have been further analysed to give the diurnal variation of occurrence of regular pulsations for quiet days only and for different seasons of the year and the results compared with a similar study of results from Tamanrasett (long. 22° 48′ N., lat. 5° 31′ E.). Two of the main deductions which may be made from this investigation, which will be reported more fully elsewhere, are: (1) that the frequency of occurrence of regular pulsations at night is greater at Legon than at Tamanrasset; and (2) that, for quiet days, in the interval 0–14 hr., the frequency of occurrence of regular pulsations at Legon is almost the same as that at Tamanrasset. In both cases there is good agreement in this time interval, with the occurrence of the phenomenon at other stations.

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Structure of the Upper Mantle as derived from the Travel Times of Seismic *P* and *S* Waves

In a recent investigation[1] I found the travel times of the seismic *P* waves (longitudinal waves) up to an epicentral distance of about 22° to conform with a structure below the Mohorovičić discontinuity that consists of an upper layer extending to a depth of about 220 km. in which the velocity is about 8·1 km./sec., increasing slightly with depth, and a lower layer in which there is a considerable increase of velocity with depth; at the boundary of the two layers there is an abrupt increase of velocity to about 8·4 km./sec. A discontinuous increase of velocity has to be assumed because the amplitudes of the waves refracted in the lower layer, observable as first arrivals from an epicentral distance of about 15°, increase gradually with epicentral distance, and at the lower end of their time-distance curve there is no focal point at which the amplitudes are very large.

The transverse *S* waves in the same distance-range are less well observed in most regions. In north-eastern America, however, good observations are obtained, and it is found that the S_d waves travelling in the uppermost part of the mantle have a velocity of about 6·5 km./sec., increasing slightly with depth[2]. They are not observed beyond about 14° epicentral distance and the *S* phases observed at greater distances are delayed. This is explainable on the assumption that there is a low-velocity layer extending from a depth of about 150 km. to the discontinuity surface at 220 km. depth. At this surface there is, as for *P*, a sudden increase of velocity and of velocity gradient.

In Europe the S_d phase of shallow shocks is so poorly observed that it was not found possible to construct a time-curve for it directly from observations. The Jeffreys–Bullen time-curve was derived from the S_d travel-times for deep-focus shocks. In a few shallow earthquakes, however, I now find the S_d phase well enough observed to be able to show that the travel times are very nearly the same as in north-eastern America.

In shallow shocks the observations of *S* at distances up to about 20° are complicated by the fact that two phases, a first small phase and a second larger one, appear at intervals ranging from a few seconds at the greater distances to 20 sec. or more at the smaller distances, and one or the other of the two phases may seem to be *S*. The first phase, which may be small enough to escape detection or may be missing, is the S_d phase. The travel times of the later phases scatter a great deal and it was not previously found possible to indicate a structure that would account for them. It appears now, however, that a structure similar to the one adopted for north-eastern America will give rise to phases having travel-times in fair agreement with those of the late *S* phases observed. From an epicentral distance of about 9° onwards they are explainable as reflexions on, and refractions in, the layer below 220 km. depth, their delay against S_d being due to the presence of a low-velocity layer. At epicentral distances smaller than 9°, second mode surface waves seem to be responsible for the late phases.

In earthquakes having their foci at intermediate depth late *S* phases also occur. The *S* phases of six Rumanian earthquakes having a common focus at 130 km. depth were examined and found to be in agreement with those calculated on the assumptions here indicated.

The travel-times of *P* were found to be consistent with a structure that had no low-velocity layer, and although it is physically possible for the velocity of *S* to decrease while that of *P* does not, yet it seems more likely that the *P* velocities are somewhat affected in the layer in which the *S* velocities are low. The velocity distribution for *P* waves was therefore altered so as to include a layer of slightly smaller velocity extending from 150 to 220 km. depth, and it was found possible to do this without altering the travel-times appreciably.

Dr. Mark Landisman of the Lamont Geological Observatory has given kind assistance in the present research, calculating travel times on the Columbia University *I.B.M.* 650 calculator.

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PHYSICS

Adsorption of Helium on Carbons: Influence on Measurement of Density

The magnitude of the adsorption of helium on solids is of interest not only theoretically, but also because of its relevance to the determination of the density of solids when helium is used as the dilatometric fluid; in such determinations the adsorption of helium is assumed to be so small as to be negligible. We have found that this assumption is not invariably correct.

In the determination of a gas/solid adsorption isotherm by use of the conventional volumetric

Table 1. HELIUM ADSORPTION COEFFICIENTS AND DENSITIES

Source of carbon	Ref. No.	Ash (per cent)	K (at 298° K. 10^{-4} ml./gm./cm. mercury)	Observed density (gm./ml.)	Corrected density (gm./ml.)
(*A*) Activated charcoals prepared from :					
Nutshell	260	3·8	7·3	2·256	1·984
Nutshell	373	5·1	6·7	2·157	1·925
Coal	372	11·1	4·1	2·145	1·999
Coal	374	6·7	4·5	2·028	1·885
Anthracite	Run 77	3·2	3·7	1·932	1·824
Anthracite	Run 78	3·8	5·2	1·994	1·829
Anthracite	Run 79	5·1	5·8	2·050	1·869
Anthracite	Run 80	6·9	6·6	2·111	1·892
Wood	403	14·0	6·4	2·395	2·125
Graphite (finely ground)	429	2·0	~7*	—	—
(*B*) Carbonized coconut shell (no activation)	310	2·5	0	1·541	1·541
Anthracite (per cent C = 93·8)	418	3·3	0	1·510	1·510
Bituminous coal (per cent C = 82·4)	346	1·25	0	1·332	1·332
Carbon black	289	0·1	0	~2·00	~2·00

* Difficult to outgas completely ; pumped at 630° K. for 1 week.

adsorption apparatus, the dead-space within the apparatus is assessed by application of the gas laws to the pressure/volume data for a given amount of helium enclosed in the apparatus. Should the adsorption isotherm of helium on the solid be linear—as is very probable—the adsorption of helium will remain undetected during the assessment of the dead space of the apparatus ; conversely, the adsorption isotherm of helium on a solid cannot be determined by conventional means. For the measurements reported here, the effect of temperature on adsorption has been employed by measuring the apparent dead-space of a conventional volumetric adsorption apparatus, the temperature of the bulb containing the adsorbent being changed after each determination. The occurrence of appreciable adsorption is shown by an increase in the apparent dead-space as the temperature of the adsorbent is lowered. Our investigations have embraced various forms of carbon as the adsorbent, and the temperature of the adsorbent has been varied over the range 77–580° K. All adsorbents (with one exception) were out-gassed at 630° K. for at least 24 hr. before adsorption measurements were made, and reproducible results were obtained with both ascending and descending temperatures.

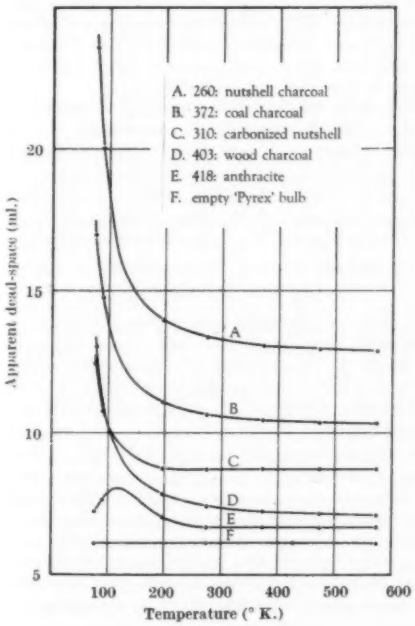

Fig. 1

Several typical sets of results are shown in Fig. 1, which is a graph of the apparent dead-space as a function of temperature. In all cases the value of the apparent dead-space became constant, or nearly so, at the higher temperatures, and the value observed at 580° K. has been taken as the true dead-space for the purpose of calculating minimum values for the adsorption of helium at lower temperatures. With all the forms of carbon examined (see Table 1) adsorption at 77° and 90° K. was readily observed and measured. At 273° K. adsorption was small and varied in extent with the type of carbon. Since our present concern lies with the influence of this adsorption on density measurements, the adsorption at 298° K. (a common temperature of operation in such determinations) is recorded in Table 1 as the slope of the linear adsorption isotherm (ml. standard temperature and pressure per cm. mercury pressure per gm. adsorbent).

On the basis of these results two groups of carbon may be differentiated.

(*A*) Carbons which absorb appreciable quantities of helium at room temperature. All the activated carbons come into this group. Results so far indicate adsorption proportional to surface area ; if the latter is assessed from determinations of the heat of wetting in methanol, an approximate guide to the extent of adsorption at 298° K. on a given carbon is given by the proportionality constant $2{\cdot}1 \times 10^{-5}$ ml. helium per cm. mercury per cal. Adsorption at room temperature was also observed on a finely divided graphite (nitrogen, Brunauer–Emmett–Teller area, 625 m.²/gm.).

(*B*) Carbons for which no adsorption at 298° K. has been detected. The following carbons are found in this group : an anthracite (C = 93·8 per cent dry ash-free) ; a free-burning bituminous coal (C = 82·4 per cent dry ash-free) (because of the continued evolution of gases with increasing temperature, the coal was outgassed at 430° K., and the dead-space measured at 398° K. was taken as the true dead-space) ; carbonized coconut shell (unactivated) ; a carbon black (particle size 190 Å., from electron micrographs).

Both the anthracite and the coal gave evidence of restricted adsorption at 77° K. (ref. 1). For group *A* carbons the effect of these small adsorptions on the measured density is most marked. The densities of

the carbons as measured by the method of helium displacement at 298° K. are given in Table 1; in the last column are the densities corrected for the observed adsorption of helium.

The reason for the difference in behaviour of the two groups of carbons is not immediately apparent. Both groups contain carbons of comparable specific area; no correlation with ash content is discernible. It appears possible that very small capillaries accessible only to helium may be present in group *A* carbons; some support is given to this explanation by density measurements using methanol as the dilatometric fluid, though this interpretation is dependent on the value assigned by Franklin[2] to the correction for adsorption of methanol.

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A Self-supporting Metal Fresnel Zone-plate to focus Extreme Ultra-violet and Soft X-Rays

In the wave-length region between 10 Å. and 1000 Å., ordinary lenses and mirrors fail as image-forming devices because the transmissivity and reflectivity of materials are very low. In this region diffraction offers a means of bending rays and focusing them.

We have produced a Fresnel zone-plate consisting of 19 metal zones held together by thin radial struts so that the transparent zones are empty. This permits soft X-rays and ultra-violet radiation to pass through and be focused. The device has been tested with visible light and ultra-violet light down to 2537 Å. It behaves as expected in terms of speed and resolution, leading to the belief that it should focus well even at wave-lengths less than 100 Å.

The zone-plate has a central circle of diameter 0·04 cm., an outer circle of diameter 0·26 cm., and is about 10 microns thick. The narrowest gold band has a width of 20 microns. It was made by the Buckbee Mears Company of St. Paul, Minnesota, by techniques that are an outgrowth of the lithographic art.

The zone-plate was better than the optimum pinhole by a factor of 6 in resolution and 40 in speed. Pictures of fine-mesh screens showed that its resolution compared favourably with that of a simple plano-convex lens of similar aperture and focal length. The lens was about 20 times faster than the zone-plate in visible light, but at 1000 Å. the zone-plate would have been much faster than a lens of any known material. Tests made at 6700, 4358 and 2537 Å. showed that the resolution improved, as would be expected from the Rayleigh criterion, auguring well for resolution at 100 Å.

Means are being considered for integrating the information from a bank of many zone-plates, so as to increase the speed of the system, possibly by some television-type scanning and integrating device.

We may gain some idea of possible future uses of a zone-plate X-ray telescope in an orbiting satellite above the Earth's absorbing atmosphere by considering the results of an experiment at 2537 Å. and extrapolating. At 2537 Å. the angular resolution was close to the theoretical value of $1 \cdot 2 \times 10^{-4}$ radian and held over a field of at least $1 \cdot 75 \times 10^{-2}$ radian, which is 2·0 times the angle subtended by the Sun's disk at the Earth.

A detailed paper on this investigation is being submitted to the *Journal of the Optical Society of America*.

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Interpretation of Intensity Distributions in the N_2 Lyman–Birge–Hopfield and CO Fourth Positive Band Systems

In much of the early spectroscopic literature the photographic density (measured or estimated by eye) of spectral features is reported, in addition to wave-lengths, as an aid to identification. In many molecular band systems in the vacuum ultra-violet region (which are becoming of increasing interest in astrophysics and plasma physics and the intensities of which, *in emission*, are relatively difficult to measure) the early data on photographic blackening are still the only experimental information on band intensities available.

Until reliable measurements of intensities have been made, it is therefore worth while to inquire into what quantitative conclusions, if any, may be drawn from the plate blackenings.

A simple method was developed some years ago[1,2] by which a test of quantitative significance is first applied to the blackening data, after which quantitative conclusions may be drawn from analysis of those data which are retained as meaningful. This method has now been applied to blackening data reported for the $N_2(a^1\Pi_g - X^1\Sigma_g^+)$ Lyman–Birge–Hopfield and the $CO(A^1\Pi - X^1\Sigma^+)$ Fourth Positive band systems which are comparable transitions of isoelectronic molecules and lie in the vacuum ultra-violet region between about 1250 Å. and 1850 Å.

It has been shown[1], for molecular band systems, that the plate blackening B_λ is related (*a*) to the emulsion response parameters: contrast γ_λ and inertia $I_{o\lambda}$; (*b*) to the molecular parameters: wave-length $\lambda_{v'v''}$, Franck–Condon factor $q_{v'v''}$, electronic transition moment $R_e(r)$, and r-centroid $\bar{r}_{v'v''}$; and (*c*) to the population $N_{v'}$ in the vibrational level v' by:

$$B_\lambda = \gamma_\lambda [\text{constant} - \log I_{o\lambda} + \log N_{v'} + \log q_{v'v''} - 4 \log \lambda_{v'v''} + 2 \log R_e(\bar{r}_{v'v''})] \quad (1)$$

The test of quantitative significance is to plot B_λ against $\log q_{v'v''} - 4 \log \lambda_{v'v''}$ for each v'' progression (v' = constant). Meaningful data fall on straight lines of roughly equal slope γ^λ for each progression. Aberrant B_λ values are rejected. The relation between $\log R_e(r)$ and $\bar{r}_{v'v''}$ may then be found by plotting $B_\lambda/\gamma_\lambda + 4 \log \lambda_{v'v''} - \log q_{v'v''}$ against $\bar{r}_{v'v''}$ (or $\lambda_{v'v''}$, as $\bar{r}_{v'v''}$ and $\lambda_{v'v''}$ are monotonically related[3]) for each progression. The segments, one for each progression, will be displaced in relative ordinate by amounts depending upon the influence of $N_{v'}$, from which the distribution of $N_{v'}$ with v' can be found. Objective rescaling of the segments leads to a graph of $2 \log R_e(r)$ + a constant versus $\bar{r}_{v'v''}$ (or $\lambda_{v'v''}$) for the entire band system.

This procedure has been carried through for the blackening B_λ and wave-lengths $\lambda_{v'v''}$ reported by Headrick and Fox[4] for the CO Fourth Positive system and by Birge and Hopfield[5] for the N_2 Lyman–Birge–Hopfield system. Franck–Condon factors were available for each system[6,7] as were r-centroids[8,9]. In the graphs actually made, however, it was sufficient to plot $B_\lambda/\gamma_\lambda + 4 \log \lambda_{v'v''} - \log q_{v'v''}$ versus $\lambda_{v'v''}$. Data were found to be meaningful for 19 bands of six progressions ($v' = 0$–5) for the Lyman–Birge–Hopfield system, and for 11 bands of four progressions ($v' = 0, 1, 3, 4$) of the CO Fourth Positive system. In both cases the graph of $B_\lambda/\gamma_\lambda + 4 \log \lambda_{v'v''} - \log q_{v'v''}$ versus $\lambda_{v'v''}$ was parallel to the $\lambda_{v'v''}$ axis, which is good evidence that $R_e(r)$ must be almost independent of r, and thus constant, for each system.

It may therefore be concluded that for these two bands systems the $(v'v'')$ array of relative vibrational transition probability :

$$p_{v'v''} = R_e^2(\bar{r}_{v'v''})q_{v'v''} \qquad (2)$$

which, influences, the intensity $I_{v'v''}$ in emission of the $v'v''$ band through :

$$I_{v'v''} \propto N_{v'} p_{v'v''}/\lambda^4_{v'v''} \qquad (3)$$

is probably, therefore, well represented in each case by the respective Franck–Condon factor $q_{v'v''}$ array.

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Millimetre Waves from Mercury Arc Harmonic Generator

The use of very small mercury arcs for the purpose of harmonic generation from a microwave input at 2·5 Gc./s. has already been described[1]. I wish now to report the preliminary results obtained with a driving frequency of 35 Gc./s.

For operation at this high frequency, the arc was formed between a vertical tungsten wire 'anode' mounted parallel to the electric vector of the microwave field across a Q-band wave-guide of stainless steel, a mercury pool cathode forming the lower surface of the wave-guide. Harmonics generated by the arc were collected by means of a smaller wave-guide protruding perpendicularly through the side wall of the Q-band guide. The whole device was filled with argon gas at 20 atmospheres pressure, mica windows permitting the entry and exit of microwave power. The level of the mercury surface could be varied by tilting, and the length of the arc was thus adjusted to be less than 0·5 mm.

The 35 Gc./s. drive was supplied by a continuous-wave klystron supplying about 1 watt into the discharge, an 8-volt d.c. supply connected across the arc causing a noticeable increase in efficiency of harmonic production. The combined rectified current was about 100 m.amp.

The highest harmonic found so far is the sixth, corresponding to a frequency of 210 Gc./s. and a wave-length of 1·43 mm., the receiver being a Golay infra-red detector. It has not yet been possible to 'match' the arc to the output circuit, so that the power reaching the detector at 210 Gc./s. is quite small (rather less than 10^{-8} watt); but if an efficient coupling can be devised it should be possible to extract up to a hundred times this power from the discharge. The power in the fourth harmonic (2·15 mm. wave-length) is about 15 db. greater than that in the sixth.

The work described here forms part of the research programme of the National Physical Laboratory, and this communication is published by permission of the Director of the Laboratory.

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METALLURGY

Crystal Growths and the Corrosion of Iron

The effect of climate on the corrosion of iron has been the subject of many studies. Hudson[1] and more recently Copson[2] have summarized studies on the rate of corrosion of ingot iron in dry and moist climates and in industrial and marine atmospheres. Ingot iron in the dry atmosphere of Khartoum, Egypt, corroded at a rate of 28 millionths of an inch a year while a specimen in the wet country atmosphere of Llanwrtyd Wells, England, corroded at a rate of 1888 millionths of an inch a year.

Critical laboratory studies by Vernon[3,4] on the effect of humidity on the corrosion of iron led to the generalization known as the "Principle of Critical Humidity". For iron, at 25° C., the rate of corrosion increases rapidly for humidities above 65 per cent. Dry oxidation is assumed to change to a rapid electrochemical attack. Local electrochemical action is assumed to occur between local anode and cathode areas through an invisible adsorbed film of water.

This model of localized electrochemical action is the basis of present-day interpretation of wet corrosion. For pure single-phase metals the sites for local cathodes and anodes have not been identified. Part of the difficulty is that liquid-phase corrosion reactions on iron are complex since hydrated oxides are often formed and the corroding metal may be removed from the reaction site.

Gulbransen and Copan[5] proposed that localized chemical reactivity in corrosion processes could be studied in gas phase reactions at elevated temperatures using more nearly ideal reaction conditions and pure metals. Since localized corrosion processes may be the result of dislocations and other defects which occur on an atomic scale, it is necessary to use high-resolution electron microscope methods.

To show the effect of water vapour on the corrosion of iron, we exposed annealed pure zone-refined iron wires to oxygen, water vapour and several gas mixtures of water vapour and oxygen. The 0·0225 cm. wires were treated in a special reactor system at 450° C. for 48 hr. For these conditions electrochemical action and solution of the corrosion product in a liquid phase are eliminated. The complex

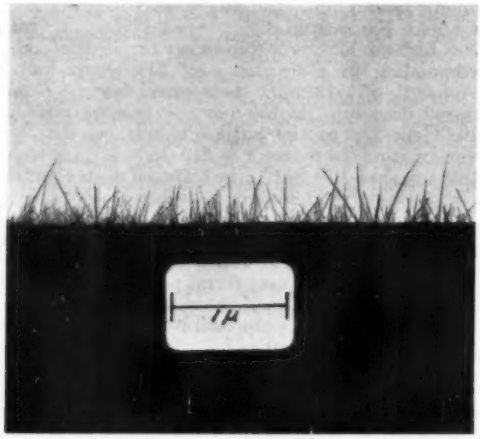

Fig. 1. Effect of moisture on crystal habits of the corrosion products formed on iron after reaction at 450° C. for 48 hr. in dry oxygen

hydrated oxides also are not stable. Electron microscope and electron diffraction studies were made of the edge of the wire specimen after reaction.

Figs. 1 and 2 shows two electron transmission micrographs of the crystal habits of the localized corrosion products. Fig. 1 shows small oxide whiskers were formed when iron was treated with dry oxygen of dew point − 79° C. These whiskers were 150–200 Å. in diameter and grew to an average height of about 8000 Å. Electron diffraction analyses showed the whiskers to be only α-Fe_2O_3. These whiskers were formed in addition to the normal oxide film. Fig. 1 suggests that the nucleation sites on the metal or on the oxide were 150–200 Å. in diameter. Since the diameter was constant along the length of the whisker, we conclude that the length of the nucleation sites remained constant as the whisker grew. The area of the nucleation site appeared to be independent of time or the extent of oxidation.

Fig. 2. Effect of moisture on crystal habits of the corrosion products formed on iron after reaction at 450° C. for 48 hr. in 10 per cent water plus 90 per cent oxygen

Fig. 2 shows the crystal habits formed when iron reacts with a 10 per cent water vapour + 90 per cent argon mixture at 450° C. for 48 hr. Many thin blade-shaped platelets were formed. These were 100–200 Å. thick, up to 8000 Å. long, and up to 70,000 Å. high. Electron diffraction analyses showed these platelets to be only α-Fe_2O_3. The surface density was estimated to be about 10^8/cm.^{2}.

The shape of the oxide platelets suggests that the site for reaction enlarges as the reaction proceeds, whereas in the iron–oxygen reaction the site remains of constant area. The constant thickness of the platelets suggests that the width of the nucleating area remains constant. One explanation for the effect of water vapour is that hydrogen ions from the water vapour act to enlarge the area for reaction.

After 48 hr. of reaction the site areas are fifty times the areas observed for the dry oxygen reaction. The total amount of oxide in the form of localized corrosion products for the water vapour reaction is about four hundred times that formed in the dry oxygen reaction.

These experiments made at 450° C. may help to explain the unique effect of water vapour on the corrosion of iron at ambient temperatures. Certain differences of course exist. The corrosion-rate will be slower, hydrated oxides may form instead of α-Fe_2O_3, the corrosion product may dissolve in the liquid phase, and electrochemical action may be present. The important factor which has not been foreseen in earlier studies is the accelerating effect of water vapour itself on localized corrosion in the absence of electrochemical action.

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ENGINEERING

Tensile Behaviour of Pyrolytic Graphite at 2,750° C.

Engineering stress–strain curves have been obtained at temperatures of 1,650–2,750° C. for a pyrolytic graphite which had been heated to 2,870° C. Changes in the crystal structure as determined by X-ray diffraction and changes in the microstructure caused by the heating and straining have been observed.

According to the manufacturer (Raytheon Manufacturing Company) the polycrystalline material tested was deposited at 2,100° C. on a substrate of synthetic graphite by decomposition of a methane–hydrogen mixture. The as-deposited material had a density-range of 2·19–2·21 gm./c.c. and, as shown in Fig. 1, had a 'block-like' structure of conical elements called 'growth cones'. From the surface appearance of the specimen blanks it was possible to divide them into four classes according to the size of the visible 'growth cones'. All specimens were heated in argon to 2,870° C., held for 5 min., and cooled to room temperature prior to testing.

The details of the testing method and equipment used have been described previously[1,2]. All specimens

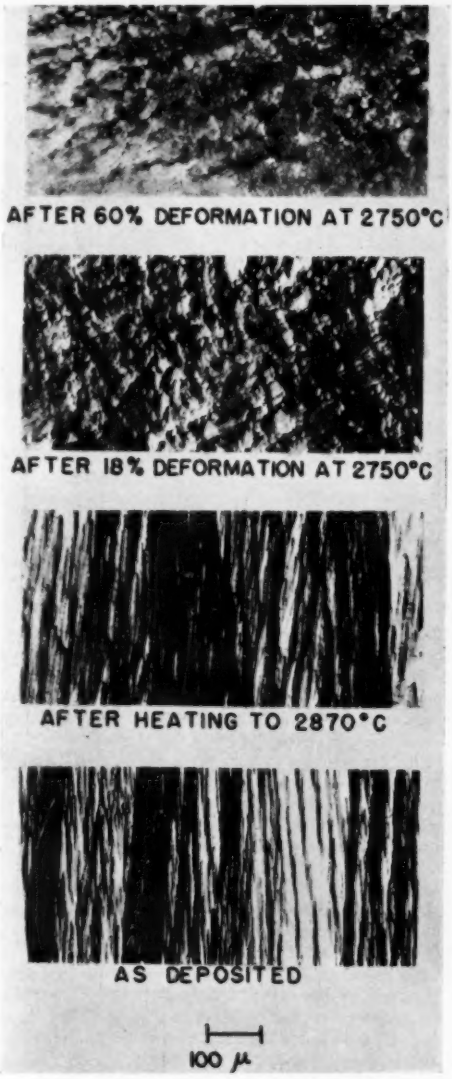

Fig. 1. Microstructure of pyrolytic graphite

had a gauge section 0·75 in. long by 0·06 in. wide by 0·1 in. thick and were taken from blanks 3 in. long by 0·75 in. wide by 0·1 in. thick with the thickness direction perpendicular to the basal planes. In some cases the as-deposited material was greater than 0·25 in. thick, so that it was possible to obtain specimen blanks 0·1 in. thick from the material deposited during the latter part of the process (Table 1 'Top'), and also from the material deposited during the early part of the process (Table 1, 'Sub'). The stress was applied parallel to the basal planes at a cross-head speed of $1{\cdot}5 \times 10^{-4}$ in./sec. All elevated temperature tests were made in helium.

Results obtained are given in Table 1. These are in general agreement with preliminary results previously reported[3]. The present specimen configuration permitted measurement of ductility and confirmed the previous indication that at temperatures above

Table 1. TENSILE PROPERTIES OF PYROLYTIC GRAPHITE

Visible growth cone size	Location* of specimen and thickness of as-deposited material (in.)	Test temperature (° C.)	Ultimate tensile strength (lb./in.²)	Elongation in 0·75 in. (per cent)
Medium	*c.* 0·17	Room	9,050	0·1
Medium fine	Top 0·25	Room	10,670	0·1
Fine	Sub 0·27	Room	9,260	0·1
Medium fine	Top 0·25	Room	6,510	0·2
Fine	Sub 0·27	1,650	19,470	4·4
Medium	*c.* 0·17	1,650	3,490†	0·1
Medium fine	Sub 0·25	1,650	15,930	4·0
Coarse	*c.* 0·10	2,200	9,300	2·8
Medium fine	Top 0·25	2,200	15,670	1·8
Fine	Top 0·27	2,200	19,420	2·8
Medium	*c.* 0·17	2,500	34,100	7·6
Medium fine	Top 0·25	2,500	34,110	5·5
Fine	Top 0·27	2,750	29,680	24·0
Fine	Sub 0·27	2,750	60,860	59·2
Fine	Top 0·27	2,750	31,600	18·2
Fine	Top 0·27	2,750	50,310	26·4

All specimens heated in argon to 2,870° C. for 5 min. and cooled to room temperature before testing.
* *c*, Centre of specimen at centre of as-deposited material; Top, specimen from top 0·1 in. of as-deposited material; Sub, specimen from bottom 0·1 in. of as-deposited material.
† Fracture occurred at growth cone.

1,650° C. pyrolytic graphite has greater ductility than commercial synthetic graphite.

The microstructure before and after deformation at 2,750° C. is shown in Fig. 1. As seen, a marked change has occurred after tensile deformation in that the 'growth cone' structure has almost disappeared. This is true after 18 per cent as well as after 60 per cent deformation and is similar to the change reported by Pappis[4]. No change in microstructure was observed to be produced by heating at 2,870° C.

Spectrometer tracings (Fig. 2) show the effects of heating and tensile deformation on the three-dimensional graphite ordering. After the as-deposited material was heated to 2,870° C. and after 18 per cent deformation at 2,750° C. there appeared to be partial

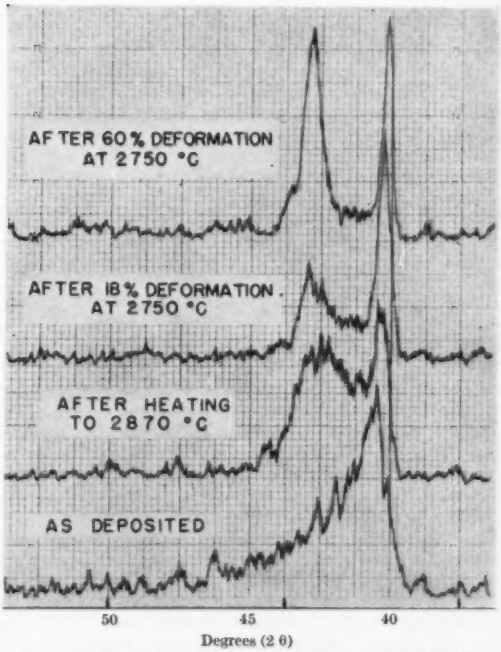

Fig. 2. Development of modulations in the (10) X-ray reflexion of pyrolytic graphite with heating and straining

three-dimensional ordering. After 60 per cent deformation at 2,750° C. there appeared to be complete ordering, as indicated by the complete splitting of the two dimensional (10) reflexion into the crystalline (100) and (101) reflexions.

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CRYSTALLOGRAPHY

Effect of a Second Crystalline Modification in Polyethylene on X-Ray Crystallinity Measurements

From several investigations it is known that in solid polyethylene which has undergone severe mechanical treatment a second crystalline modification may occur besides the normal rhombic one[1]. In the X-ray diagram the strongest reflexion of this second modification coincides with the maximum in the so-called amorphous band (the broad halo of diffraction emanating from the amorphous regions). In view of the fact that the amorphous band in unbranched polyethylenes appears to be abnormally narrow, Slichter[2] suggests that the second modification also occurs in undeformed polyethylene. In this communication I want to point out that this suggestion may explain certain anomalies in the results of the density and crystallinity measurements described below.

If polyethylene is assumed to consist of an amorphous and a rhombic crystalline phase only, the density d_{tot} is related to the crystalline fraction φ as follows:

$$\frac{1}{d_{tot}} = \frac{\varphi}{d_{rh}} + \frac{1-\varphi}{d_{am}}$$

where d_{rh} and d_{am} are the (rhombic) crystalline and amorphous densities, respectively. Originally, our aim was to find d_{am} values for different specimens from measurements of the quantities d_{tot}, d_{rh} and φ. The measurements were made as follows: (1) d_{tot} was determined at 23° C. by a flotation method. (2) d_{rh} was calculated from the cell dimensions, which were determined from the X-ray diffraction angles of the (200), (020) and (011) reflexions. The polyethylene samples were mixed with sodium chloride which served as a standard. (3) The crystalline fractions were measured by Krimm and Tobolsky's[3] X-ray method. In this method the intensity of coherent scattering from the amorphous fraction is compared with the corresponding intensity from the molten sample. The necessary monochromatization of the X-ray beam was obtained by the double filter technique of Ross[4] as described by M. Kakudo and R. Ullman (private communication).

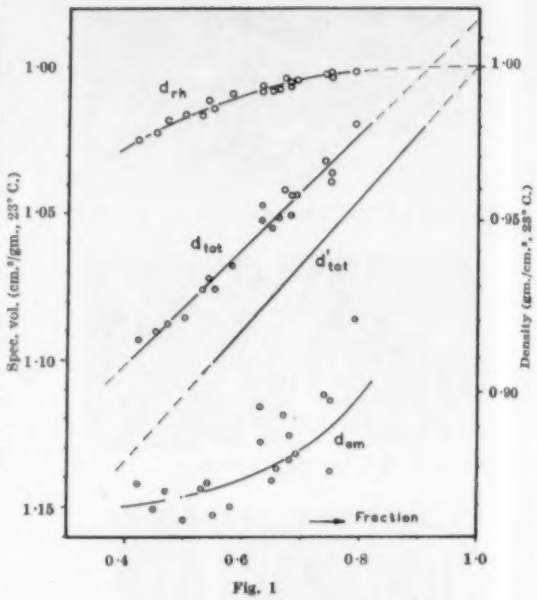

Fig. 1

Altogether five different polyethylene samples were investigated, each being cooled from the melt at four different rates.

In Fig. 1 both the measured values of d_{rh} and d_{tot} and the calculated values of d_{am} are presented as functions of the crystallinity φ. (The curve through the points d_{tot} is based on some twenty more incidental measurements, which are not shown in the figure.)

This graph is not satisfactory for two reasons. First, the calculated values of d_{am} are in the range 0·865–0·92 gm./cm.³, which is much higher than that found by extrapolating the density of molten polyethylene to room temperature (0·83–0·85 gm./cm.³). The maximum density to be expected for amorphous polyethylene can be estimated by calculating the density of a hypothetical phase in which the chain molecules are parallel and close-packed, and can rotate around their chain axes. With the shortest possible distance between the centres of the chains (4·95 Å.), the calculated density is 0·86 gm./cm.³, which is lower than the values shown in Fig. 1.

Secondly, the extrapolated curves for d_{tot} and d_{rh} should intersect at $\varphi = 1$, which they clearly do not.

A possible explanation of these discrepancies is that some systematic error was made in our measurements. This explanation seems to be supported by the fact that the density-crystallinity relation d'_{tot} (Fig. 1), which was obtained in our laboratory from infra-red measurements, leads to much higher crystallinities and can moreover be extrapolated to intersect the d_{rh}-curve at $\varphi = 1$. The line d'_{tot} also agrees with nuclear magnetic resonance measurements made in our laboratory of the 'mobile' fraction in different polyethylene samples at room temperature.

However, the differences between the amorphous fractions following from the lines d_{tot} and d'_{tot}, respectively, seem to be too large to be attributable

to experimental errors alone. We believe that the discrepancies must be explained by the above-mentioned suggestion, namely, that also in normal polyethylene a second crystalline modification is present. In the X-ray method this fraction is 'seen' as amorphous, in the other methods as crystalline.

This view is supported by measurements of the integral breadth of the amorphous band, from which it followed that for highly crystalline samples this breadth is only about two-thirds of the value found for molten polyethylene. This agrees with the idea that a (perhaps highly broadened) crystalline peak is hidden under the amorphous band.

If this explanation is assumed, the fraction φ_2 of the second modification may be roughly estimated to correspond with the horizontal distance between the lines d_{tot} and d'_{tot}. Using for the density d_2 of the second modification the value suggested by Teare and Holmes (0·965 gm./cm.³, ref. 1), the amorphous density may now be calculated anew from the relation :

$$\frac{1}{d_{tot}} = \frac{\varphi_{rh}}{d_{rh}} + \frac{\varphi_2}{d_2} + \frac{1 - \varphi_{rh} - \varphi_2}{d_{am}}$$

This leads to values of about 0·84 gm./cm.³ for all samples, which is in good agreement with the range of values found by extrapolation of the density of molten polyethylene.

I am indebted to Dr. Westrik for many helpful suggestions. The density, infra-red, and nuclear magnetic resonance measurements were made by Dr. Fortuin, Dr. Veerkamp and Dr. Smidt, respectively, who kindly gave me permission to use their results in this discussion.

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CHEMISTRY

Preparation of Pure Standard Metallic Perchlorate Solutions

Acid-free standard solutions (0·05 *M*) of the perchlorates of the divalent metals copper, nickel, cobalt, iron, zinc and manganese suitable for use in the determination of stability constants of chelates by the potentiometric method can be prepared utilizing an ion-exchange technique. A column 1·6 cm. diameter containing 50 ml. 'Amberlite' cation exchange resin *IR* 120(*H*) is treated with a solution (0·1 *M*) of the pure sulphate or chloride of the divalent metal until the *p*H's of the entrant and issuing solutions are identical. After eluting the excess metal salt solution with water the metal ions bound to the resin are displaced by passing a pure solution of barium perchlorate (0·052 *M*) on to the resin. Since barium ions have a stronger affinity for the resin than the divalent ions of copper, nickel, cobalt, iron, zinc and manganese, the latter are displaced and the barium-free fractions of the eluates contain the pure perchlorate solutions of these metals. A yield of about 400 ml. 0·05 *M* metal perchlorate solution is obtained under these conditions. An analysis of each of the solutions was carried out using both gravimetric and ion exchange methods. In the latter a 20-ml. portion of the metal was passed on to a small column of 'Amberlite' resin *IR* 120(*H*) and the eluate titrated with standard alkali. The results obtained by the two methods agreed to within 0·3 per cent.

The ferrous perchlorate solution can be conveniently stored in the ion exchange column which, for ease of dispensing, is connected to a burette fitted with an oxygen trap. The solution should be standardized immediately before use and contains less than 10 p.p.m. Fe^{3+}. In order to obtain a maximum yield it is necessary to exclude air from the solutions used for the quantitative conversion of the resin into the form $(RSO_3)_2Fe$. However, while the yield is lowered by the presence of Fe^{3+} on the resin the purity of the ferrous perchlorate is not affected since during the subsequent treatment with barium perchlorate only ferrous ions are displaced from the resin.

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Catalytic Effects in the Alkaline Oxidation of Cellulose

Michie and Neale[1] have reported results which suggest that copper has an unexpectedly high catalytic effect in the reaction of cellulose with oxygen in the presence of low concentrations of alkali. By comparing their observed rate of reaction for copper-treated cotton containing fully carbonated sodium hydroxide with a value calculated from Davidson's[2] results for the uncatalysed reaction at high sodium hydroxide concentration, they deduced that the copper increased the rate by a factor of 80 or more. Very much smaller increases, namely, less than ten-fold, had been found by other workers, concerned with alkali concentrations of 5 *N* or higher, on addition of metallic catalysts.

A more direct demonstration of a large catalytic effect at fairly high alkali concentration is provided by the results in Fig. 1, which shows the variation of the initial rate of oxygen consumption of jute fibre at 60° C. with the iron content of the fibre. The

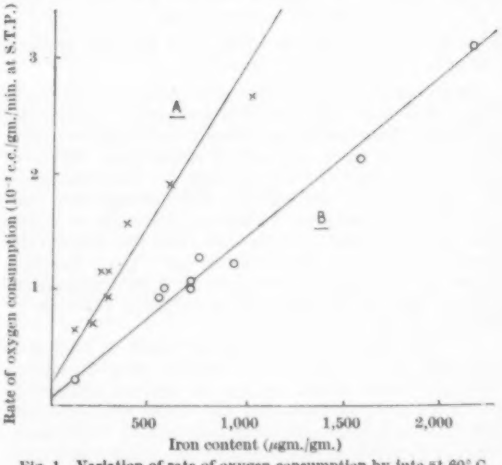

Fig. 1. Variation of rate of oxygen consumption by jute at 60° C. with iron content of fibre

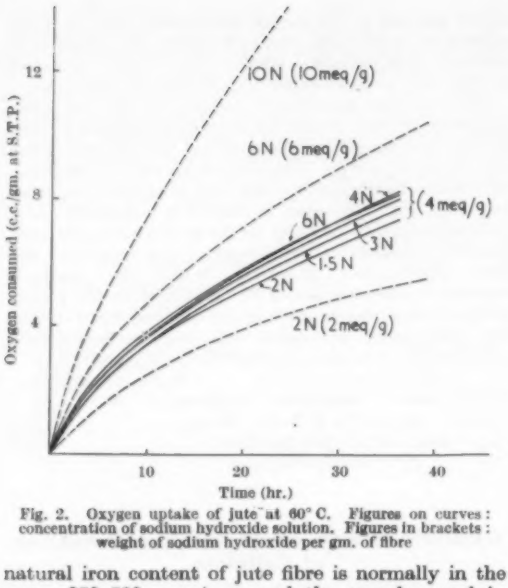

Fig. 2. Oxygen uptake of jute at 60° C. Figures on curves: concentration of sodium hydroxide solution. Figures in brackets: weight of sodium hydroxide per gm. of fibre

natural iron content of jute fibre is normally in the range 250–500 μgm./gm., and the samples used in these experiments included untreated fibre, fibre treated with acid to reduce the iron content, and fibre impregnated with different amounts of ferric sulphate. The rate at which oxygen was consumed by the fibre was determined from the fall in pressure in a closed vessel, filled with pure oxygen, in which the powdered fibre, moistened with a measured volume of sodium hydroxide solution, was spread over the inner surface. The oxygen uptake was normally linear with time over the first few hours and thereafter gradually decreased. An induction period, such as found by Davidson[2] for cotton, was not observed with jute fibre. The rates plotted in Fig. 1 were estimated from the slope of the initial linear portion of the uptake curves. The two lines, *A* and *B*, are drawn through points for two sets of experiments in which different quantities of sodium hydroxide were added to the fibre, as follows:

A, 2·67 ml. 3 *N* sodium hydroxide per gm. of fibre (8 m.equiv. sodium hydroxide/gm.).

B, 1·33 ml. 3 *N* sodium hydroxide per gm. of fibre (4 m.equiv. sodium hydroxide/gm.).

These results demonstrate two points of interest:

(*a*) Over the range of iron contents covered, the rate of reaction increases linearly with the iron content. In the results of set *B* a catalytic factor of about 40 is involved on increasing the iron concentration from zero to the highest experimental iron content of 2,170 μgm./gm. Other experiments at higher iron contents, however, showed that the linear relation did not hold above 2,000 μgm./gm., the rate apparently becoming independent of iron concentration, in agreement with the conclusion reached by Michie and Neale[1].

(*b*) The ratio of the slopes of lines *A* and *B* is very close to 2·0, indicating that the rate of reaction is proportional to the weight of sodium hydroxide per unit weight of fibre and not to the concentration of the sodium hydroxide solution applied to the fibre. This relationship is confirmed by the results in Fig. 2, which shows the uptake of oxygen with time of samples of natural jute fibre moistened with different volumes of sodium hydroxide solutions of various concentrations. The group of solid lines, for a constant weight of 4 m.equiv. sodium hydroxide per gm. of fibre, indicates a rate of oxygen consumption that is constant within the reproducibility of these experiments, although the concentration of the alkali solutions varies from 1·5 *N* to 6 *N*. If this relationship applies to cotton as well as to jute, the finding of Michie and Neale[1] that the rate of oxidation is to a first approximation proportional to the sodium hydroxide concentration would seem to be valid only if equal volumes of alkali solution were applied to a given weight of fibre.

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Infra-Red Spectra of Deoxyribonucleic Acids from Human Leucocytes

As part of a study on the physico-chemical behaviour of deoxyribonucleic acid obtained from human leukæmic leucocytes, we have examined the infra-red spectra of many normal and leukæmic samples in solutions in water and deuterium and in the dry state. Since we did not find complete agreement between previous reports of the infra-red spectra of calf thymus deoxyribonucleic acid[1–3], we have also studied samples from this source. The effects of heating, *p*H changes, degradation with deoxyribonuclease, mild and strong acid hydrolysis were examined and the spectrum of apurinic acid has also been determined.

A Perkin–Elmer, model 21, double-beam spectrometer with sodium chloride and calcium fluoride optics has been used. With slit-widths narrower than those used by Blout and Lenormant[4] and with suitable adjustments, we were able to improve the resolution. The deuterium solutions were prepared in the manner described by previous authors[4].

The samples were examined in demountable cells of thallium bromo-iodide with spacers of 0·015 mm. In order to avoid the small lack of compensation due to absorption by atmospheric water in studying details of the bands in the region between 1,700 and 1,500 cm.$^{-1}$, we flushed the spectrometer with dry nitrogen.

The spectra were observed in the regions: dry state, 4,500–650 cm.$^{-1}$; deuterium solution, 2,000–650 cm.$^{-1}$; water solution, 1,500–850 cm.$^{-1}$. All samples of deoxyribonucleic acid from normal and leukæmic sources had identical spectra. The physical or chemical treatments resulted in the same spectral modification for all samples.

The spectrum of deoxyribonucleic acid in deuterium solution, in the region between 1,700 and 1,500 cm.$^{-1}$, shows the following bands: 1,575 cm.$^{-1}$(*w*) with a shoulder at 1,563 cm.$^{-1}$; 1,622 cm.$^{-1}$(*m*); 1,645 cm.$^{-1}$ strong and sharp; 1,680 cm.$^{-1}$ strong and broad.

These results are in agreement with those obtained by DeLozé[3] for calf thymus deoxyribonucleic acid at 100 per cent relative humidity in deuterium, except that we did not find the band at 1,695 cm.$^{-1}$ in any sample.

The physical and chemical treatments mentioned above induce no change in the frequency or shape of the band at 1,575 cm.$^{-1}$, though the band becomes

stronger at *p*H 13. This band does not appear in the spectrum of apurinic acid. The band at 1,622 cm.$^{-1}$ has been found in the spectra of all samples: it became sharper after treatment with deoxyribonuclease or upon change of *p*H. For all samples, the band at 1,645 cm.$^{-1}$ completely disappears after acid hydrolysis or treatment with deoxyribonuclease and is replaced by a strong band at 1,660 cm.$^{-1}$. On heating at 100° the band at 1,660 cm.$^{-1}$ becomes broader. The band at 1,680 cm.$^{-1}$ which is strong and broad in the original spectrum decreases considerably, but does not disappear on treatment with enzyme, acid hydrolysis or on heating and there is a frequency shift to 1,690 cm.$^{-1}$. The spectrum of all samples of *p*H 13 shows a new weak band at 1,592 cm.$^{-1}$.

These results are of particular interest in relation to the nature of the specific interaction between purine and pyrimidine units in deoxyribonucleic acid structure[6-9].

Our observations are in disagreement in some respects with previous reports[1], and the explanation of the spectral changes is still open to discussion. Further data are required, and a more detailed study of the spectra changes of deoxyribonucleic acid under different *p*H conditions is now being carried out. The results and a proposed interpretation will be published elsewhere.

In previous determinations of infra-red spectra of deoxyribonucleic acid in mineral oil suspension, preparations of myeloid leukæmia leucocytes were found to exhibit a band which was not present in preparations from normal leucocytes[5]. The results of the present work, however, do not confirm this observation.

We are indebted to Dr. E. Mantica for his interest and helpful discussion.

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[For "deuterium" read "deuterium oxide" throughout.—Editors.]

Use of Solvents containing Ethyl Iodide in the Investigation of Phosphorescence Spectra of Organic Compounds

M. Kasha[1] has suggested that the yellow colour produced when α-chloronaphthalene and ethyl iodide are mixed is due to the enhancement of the lowest singlet–triplet absorption band of the naphthalene molecule. He interpreted this effect in terms of a collisional perturbation of spin-orbital coupling in the aromatic molecule, by the heavy iodine atom. The singlet–triplet absorption bands reported for benzene by a number of workers[2] as well as those for a wide number of other aromatic molecules have been shown by Evans[3] to be due to spin orbit perturbation by the inhomogeneous fields of dissolved paramagnetic oxygen molecules. The perturbing effects of ferric and cupric acetonyl acetonates have also been demonstrated[4].

It was thought that a similar effect of perturbation might be observed during phosphorescent emissions from organic compounds, both by increasing the likelihood of phosphorescent return to the ground-state, and by increasing the conversion from the excited singlet to triplet states. The effect of ethyl iodide on the emissions of the eight isomeric dinitronaphthalenes, and on N,N-dimethyl-4-nitroaniline, coumarin and acid fluorescein was investigated. The phosphorescence spectra were first measured with the compounds dissolved in a glass made by cooling a mixture of four parts of ethanol to one of methanol to the temperature of liquid air. The spectra were then remeasured in a glass containing one part of ethyl iodide to four parts of mixed alcohols. The apparatus used was a high-pressure a.c. mercury arc lamp fed through a rectifier, and a glass prism spectrometer with a photomultiplier at the exit slit. Phosphorescence was identified and recorded by 'chopping' the photomultiplier current supply so that the sample emission was only recorded during the ½ a.c. cycles when the lamp arc intensity was zero. The ratio of the intensities of the readings obtained for a given band with and without the 'chopper' was approximately 1 : 2 for long-lived phosphors. This is because the total luminescence involved viewing for twice the time available for phosphorescence readings. For short-lived phosphors, this ratio was smaller due to the rapid decay of the emission with respect to the time of an a.c. cycle. The results of these investigations are shown in Table 1. 1,5-Dinitronaphthalene is quoted as typical of the dinitronaphthalene series.

Table 1

Compound	Total luminescence : phosphorescence ratio		Life-time (sec.)	
	Alcohol	Alcohol + Et I	Alcohol	Alcohol + Et I
1,5-Dinitronaphthalene	1·97	2·28	> 0·1	0·008
N,N-Dimethyl-4-nitronaphthalene	1·97	2·32	> 0·1	0·008
Coumarin	—	2·94	> 0·1	0·005
Acid fluorescein	—	2·35	> 0·1	0·007

In all cases the effect of the addition of ethyl iodide was to increase the intensity of the phosphorescence bands and increase the ratio of the total luminescence to phosphorescence. Since the phosphorescence intensity did not appreciably decrease, the effect of the iodide on the ratio was not merely to increase the radiationless deactivation to the ground-state, but must also have increased the probability of the triplet–ground singlet transition. This evidence would seem to show that even in a rigid glass solvent 'heavy atom' perturbation occurs, thus providing the inverse effect of Kasha's observations.

Two other effects are of interest: first, the phosphorescence band peaks were shifted towards lower energies by about 200–300 cm.$^{-1}$; and, secondly, the coumarin and acid fluorescein fluorescence intensities were also increased. It is possible that some form of complex might lead to charge transfer effects

such as those reported by Reid[5]. These points will require further investigation.

It should be noted that, although ethyl iodide is well known to undergo photolysis in strong ultraviolet light, no appreciable decomposition was found under the conditions of the present work.

We wish to thank Dr. E. J. Bowen for help and encouragement. This work was carried out during the tenure of a maintenance grant from the Department of Scientific and Industrial Research by one of us (J. M. C.).

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Rate of Ligand Exchange with its Metal Complex by a Polarimetric Method

Optically active 1,2-propylenediamine tetraacetic acid[1] (H_4PDTA) co-ordinates in a completely stereospecific manner[2] with Co(III), Rh(III) and Cr(III). Courtauld models of either co-ordinated optical isomer show the methyl group to be sterically hindered for one configuration of the metal complex irrespective of whether the ligand is attached at 4 (non-planar), 5 or 6 points to the metal atom. The absolute stereospecificity of complex formation provides a simple polarimetric method of measuring the exchange-rate of the free ligand with its metal complexes.

Equivalent amounts of the metal complex containing one optical form of the ligand and the free optical antipode were mixed in buffered aqueous solution, and the rate of loss of optical activity determined.

$$[M\ d\text{-PDTA}]^{n-} + l\text{-PDTA} \rightleftarrows [M\ l\text{-PDTA}]^{n-} + d\text{-PDTA}$$

It will be evident that the rotation will have fallen to zero when half the ligand is exchanged. The concentrations of unreacted initial complex and the added free ligand at any time may be calculated from the observed rotation and the respective initial rotations before mixing. In general, there is a considerable difference between the rotatory powers of the free ligand and the metal complex even when the latter is colourless. The difference may be magnified by a suitable choice of the wave-length of the light when the complex is coloured.

Preliminary studies of the rate of exchange of the cadmium complex with the ligand have shown a first-order dependence on both complex and ligand at *p*H 6. The approximate specific rate constant at 20° C. was found to be 0·07 l.-moles^{-1}-sec.$^{-1}$. During the reaction, the rotation changed from − 0·66° to zero for 0·005 *M* solutions of complex and ligand in a 20-cm. tube.

Table 1

Complex	*p*H	Time for half-exchange with *l*-PDTA
[Ni(*d*-H_2.PDTA)]	0·70	30 min.
	2·90; 6·40	No exchange in 3 days
[Cu(*d*-H_2.PDTA)]	2·90	< 1 min.
	6·40	16 hr.
[Cd(*d*-H_2.PDTA)]	2·90	< 40 sec.
	6·30	1 hr.
[Zn(*d*-H_2.PDTA)]	2·90	< 40 sec.
	6·32	3 hr.
[Ca(*d*-H_2.PDTA)]	7·30	< 1 min.
[Al(*d*-H.PDTA)]	2·90	20 hr.
[Y(*d*-H.PDTA)]	2·90	< 1 min.
[Mn(*d*-H_2.PDTA)]	3·00	< 1 min.
	6·30	5 min.
[Co(*d*-H_2.PDTA)]	2·90	< 1 min
	6·30	7 days
[Fe(*d*-H.PDTA)]	0·7	7 hr.
	6·5	No exchange in 2 days

The approximate times for exchange between half of the PDTA and a number of its metal complexes at 20° C. are shown in Table 1. The metal complexes were prepared in the acid forms, [*M*(H_2.PDTA)], or [*M*(H.PDTA)] by reaction between the metal acetate and active propylenediamine tetraacetic acid and, after recrystallization, dissolved in the appropriate buffer solution. The complexes like those with ethylenediamine tetraacetic acid are either quinquidentate or sexadentate. Detailed studies will be published later.

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BIOCHEMISTRY

Specific Detection of Glucose on Paper Chromatograms

Mixtures of monosaccharides derived from complex polysaccharides often present difficulties in identification when the compounds migrate close to one another on paper chromatograms. Difficulties of this kind have been encountered with certain bacterial polysaccharides containing glucose, galactose and heptoses. With the use of the glucose oxidase[1]-indicator reagent, 'Glucostat' (Worthington Biochemical Corporation) it has been possible to locate specifically the glucose and then reveal other monosaccharides on the same paper chromatogram.

Monosaccharides were separated on Whatman paper No. 1, with the *n*-butanol–pyridine–water (6 : 4 : 3 v/v) solvent system, the paper chromatograms dried in air and then sprayed with the 'Glucostat' reagent prepared as for the quantitative estimation of glucose in biological fluids[2]. As the 'Glucostat' reagent is in the form of a buffered, aqueous solution, flooding of the paper chromatograms was avoided to prevent spreading of the various monosaccharides. When sugars were separated with acidic solvents such as *n*-butanol–acetic acid–water (6 : 1 : 2 v/v) the chromatograms were steamed for 5–10 min. before spraying. The indicator dye, *o*-dianizidine, in the 'Glucostat' reagent locates the glucose by turning a permanent pinkish-brown colour. The colour develops rapidly at room temperature and no further treatment is necessary. The method readily detects 10–20 μgm. glucose separated

on paper chromatograms and will detect less than 10 μgm. providing little spreading of the 'spot' occurs during chromatography. Attempts to adapt the method for direct quantitative estimation have been unsuccessful, as the coloured complex adheres strongly to the paper and could not be extracted quantitatively in a variety of solvents. Thus, for determination of glucose, it is necessary to elute after separation on paper chromatograms and then develop the colour in the normal manner with the glucose oxidase–*o*-dianisidine reagent[2].

After detection of glucose, the paper chromatograms were dried in air and over-sprayed with aniline phthalate for the identification of other monosaccharides. There is some loss in sensitivity towards reaction with the aniline phthalate, presumably due to the buffer constituents, protein, etc., of the 'Glucostat' reagent. However, for differentiation between glucose and other monosaccharides poorly separated from the former sugar, spraying first with the glucose oxidase reagent and then over-spraying with aniline phthalate provides a rapid and reproducible technique. The recent report of a galactose oxidase[3] suggests that such an enzyme could also be applied in a similar fashion for the specific identification of galactose.

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Glutathione Stability and Pyrophosphatase Activity in Reticulocytes; Direct Evidence for the Importance of Glutathione for the Enzyme Status in Intact Cells

In the course of studies on the maturation of red blood cells we confirmed the report of Malkin and Denstedt[1] that reticulocytes show a tenfold higher activity of inorganic pyrophosphatase as compared with erythrocytes. Pyrophosphatase appears to contain a functional SH— group. Traces of cupric ions, *para*-chloromercury benzoate and iodo-acetate cause an inhibition which is reversible by cysteine[2,3]. To test the assumption that the lability of the SH— group may be connected with the decrease of pyrophosphatase activity during maturation, the effect of conditions which change the oxidation status of glutathione on the activity of pyrophosphatase was studied.

The experiments were performed on intact cells and hæmolysates of rabbit blood, in which a reticulocytosis of 35–40 per cent was brought about by bleeding. Table 1 contains the results of an experiment on intact cells in which phenylhydrazine was employed to oxidize the glutathione according to Beutler's procedure[4]. It may be seen that incubation of washed cells without phenylhydrazine did not occasion a decline of pyrophosphatase activity; this did occur in the presence of phenylhydrazine. Addition of glucose prevented the loss of enzyme activity. In Table 2 is shown a similar experiment on stroma-free hæmolysate. Here the loss of enzyme activity could be prevented by the addition of glucose-6-phosphate. The mechanism of the action of phenylhydrazine is in all likelihood a hæmin-catalysed oxidation of glutathione[5,6]. The conservation of glutathione with glucose or glucose-6-phosphate occurs by way of the chain of enzymes hexokinase, glucose-6-phosphate-dehydrogenase and glutathione reductase[7].

Table 1

	I	II	III	IV
Incubation time (hr.)	0	10	10	10
Phenylhydrazine (5 mgm./ml. susp.)	—	—	+	+
Glucose (2×10^{-2} *M*)	—	—	—	+
Pyrophosphatase units	22·1	21·4	9·5	19·8

The reaction system for the determination of pyrophosphatase activity contained in 2·0 ml.: 1×10^{-2} *M* $Na_4P_2O_7.10H_2O$, 2×10^{-2} *M* $MgSO_4.7H_2O$, 1×10^{-1} *M tris*-buffer *p*H 7·6, 0·1 ml. hæmolysate. Incubation for 15 min. at 37° C. The reaction was stopped by precipitation with trichloracetic acid. The liberated inorganic phosphate was measured by the method of Fiske and Subbarow (ref. 11). Pyrophosphatase activity is reported as γ phosphorus liberated/ml. reaction mixture.

Table 2

	I	II	III	IV
Incubation time (hr.)	0	4	4	4
Phenylhydrazine	—	—	+	+
Glucose-6-phosphate (5×10^{-3} *M*)	—	—	—	+
Pyrophosphatase units	25·9	22·1	8·2	22·1

In the following experiments the direct effect of oxygen was studied and further evidence for the depicted course of events was sought. Table 3 records an experiment in which hæmolysates with and without stroma, which had been dialysed for 60 hr., were incubated. It may be seen that oxygen alone in the presence of stroma caused more than 85 per cent loss of enzyme activity (II). The addition of glutathione alone (IV) diminished the inactivation only to a small extent. Addition of glucose-6-phosphate (VII) also showed only incomplete stabilization of pyrophosphatase. In the presence of nicotinamide (VI), presumably by virtue of inhibition of triphosphopyridine-nucleosidase[8], a higher degree of stabilization was observed. In a complete system (V), complete stabilization was achieved. In the absence of stroma the decrease of enzyme activity was much less (2), and the addition of glutathione alone (4) was sufficient for stabilization. The experiments not only prove that oxygen without intervention of phenylhydrazine may oxidize glutathione but also show that stroma, probably by way of autoxidation of the unsaturated fatty acids, combined with the action of hæmin[9], may favour this oxidation. In addition, stroma increases the inactivation by way of destruction of triphosphopyridine nucleotide through its enzyme. The partial stabilization of pyrophosphatase without addition of triphosphopyridine nucleotide is in consonance with the report of Carson[10], which showed the triphosphopyridine nucleotide is tightly bound to glucose-6-phosphate dehydrogenase.

Table 3

	Complete hæmolysate							Stroma-free hæmolysate			
	I	II	III	IV	V	VI	VII	1	2	3	4
Incubation time (hr.)	0	6	6	6	6	6	6	0	6	6	6
Glutathione ($2 \cdot 5 \times 10^{-3}$ *M*)	—	—	—	+	+	+	+	—	—	—	+
Glucose-6-phosphate (5×10^{-3} *M*)	—	—	+	—	+	+	+	—	—	+	—
Triphosphopyridine nucleotide ($1 \cdot 5 \times 10^{-4}$ *M*)	—	—	+	—	+	—	—	—	—	+	—
Nicotinamide (5×10^{-2} *M*)	—	—	+	—	+	+	—	—	—	+	—
Pyrophosphatase units	30·8	4·3	28·2	9·5	28·6	17·8	11·5	30·0	19·3	28·8	28·6

Table 4

	I	1	II	2	x^*	y^*
Incubation time (hr.)	0	0	6	6	12	12
Cysteine (2×10^{-4} *M*)	—	+	—	+	—	+
Pyrophosphatase units	13·2	16·5	6·6	12·7	1·2	2·9

* *x*, *y* from another experiment, activity without incubation, 18·7.

In Table 4 is shown the fact that the inactivation of pyrophosphatase is to a large extent reversible by addition of cysteine after a short period of incubation. The conclusions of these experiments may be summarized as follows.

The stability of pyrophosphatase in the reticulocytes is closely linked with glutathione, which is maintained in the reduced state by way of the oxidation pathway of glucose-6-phosphate. Eliminations or blocking of any single step in the chain may cause a stoichiometric oxidation of glutathione with conequent loss of activity of pyrophosphatase.

Oxidation of glutathione leads to a denaturation of pyrophosphatase, the first reversible step of which is formation of disulphide-bonds. Thus there is close interaction between metabolic processes, such as are concerned with the maintenance of glutathione, and the structural changes of denaturation.

The experiments reported are, so far as we know, the first instance of a direct effect of glutathione on the activity of an enzyme within an intact cell. Other SH— enzymes are in progress.

The mechanism described may have general importance for the conservation and the decline of enzymes in the processes of differentiation and maturation of cells.

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Characterization of a New Purine Riboside isolated from *Fusarium* Species

An investigation of the acid-soluble nucleotides occurring in a *Fusarium* species[1] has shown that, in addition to a number of previously known compounds, several new ultra-violet absorbing substances are present in this mould. One of these was identified as the mononucleotide of nicotinic acid[2], a compound which has also been isolated from baker's yeast[3]. The present communication reports the characterization of a second substance, an N^2-substituted guanosine which is probably 2-(1-carboxyethylamino)-6-hydroxy-9-β-D-ribofuranosylpurine.

The compound was isolated from perchloric acid extracts of the micro-organism by ion exchange chromatography[4] and purified by paper chromatography. From a 50-l. culture grown in a stirred fermentor[5] on Czapek–Dox medium 2·5 μmoles were obtained, as calculated from the pentose content of the chromatographically pure substance. The product was phosphorus-free and had ultra-violet absorption spectra at different *p*H values very similar to those of guanosine, except for a small bathochromic shift of the maxima and the minima. Paper electrophoresis in 0·1 *M* ammonium acetate, *p*H 6·5, indicated the presence of a negatively charged group. The orcinol reaction for pentoses was positive; the pentose was liberated on acid hydrolysis (2 hr. at 100° C. with 1 *M* hydrochloric acid) and identified as ribose by paper chromatography in *n*-butanol–acetone–water (2 : 7 : 1, v/v/v). The ribose moiety of the new compound had the furanosidic structure, as shown by the formation of glycerol after degradation according to the procedure of Viscontini, Hoch and Karrer[6] scaled down to 0·3 μmole[7]. Acid hydrolysis yielded, in addition to ribose, an ultra-violet absorbing compound which was purified by ion exchange and paper chromatography. It gave a negative reaction for pentoses, had ultra-violet spectra at acid, neutral and alkaline *p*H values nearly identical to those of 6-hydroxy-2-methylaminopurine[8], and paper electrophoresis indicated the presence of an acidic group. When heated with 4 *N* formic acid (15 hr. at 125° C.) it yielded guanine, which was identified by paper chromatography in two solvent systems and by its ultra-violet spectrum.

These results strongly suggested that the compound isolated from the mould was the ribofuranoside of an N^2-substituted guanine containing an acidic group. The close similarity of the ultra-violet spectra of the hydrolysed compound to those of 6-hydroxy-2-methylaminopurine indicated that the acidic group was most likely carried by the N^2-substituent, so that the aglycone would be an N-(6-hydroxy-2-purinyl)-amino-acid. To test if this were correct a number of compounds of this type were synthesized by treating at *p*H 8·5–9·0 aqueous solutions of 2-chloro-6-hydroxypurine[9] with each of 22 amino-acids in sealed tubes for about 15 hr. at 110–125° C. The amino-acids used were α- and β-alanine, α-, β- and γ-aminobutyric acid, ε-aminocaproic acid, β-amino-*iso*butyric acid, aspartic acid, glutamic acid, glycine, *iso*leucine, leucine, methionine, S-methylcysteine, norvaline, phenylalanine, sarcosine, serine, taurine, threonine, tyrosine, valine. The ultra-violet spectra of the synthetic compounds were practically identical to those of the natural aglycone, except for that of the compound prepared from sarcosine, which showed a remarkable bathochromic shift of the maxima at acid and neutral *p*H values (a similar behaviour is shown by 2-dimethylamino-6-hydroxypurine, as compared with 6-hydroxy-2-methylaminopurine[8]) and for that of the compound prepared from tyrosine, which is strongly influenced by the amino-acid chromophore.

When the paper electrophoretic and chromatographic mobilities of the synthetic compounds were determined, a definite correlation with some chemical features of the N^2-substituent was found. Among the whole set of model substances prepared, only those obtained by treating 2-chloro-6-hydroxypurine with α-aminomonocarboxylic acids behaved electrophoretically as the natural purine. This, moreover, could be distinguished from all of the synthetic compounds, except for the α-alanine and threonine derivatives, by paper chromatography in two solvent systems. In order to decide which, if either, of these two products was identical with the aglycone of the ribo-

Table 1. PROPERTIES OF THE RIBOSIDE ISOLATED FROM *Fusarium* SPECIES, CORRESPONDING AGLYCONE, AND SYNTHETIC 6-HYDROXY-(1-CARBOXYETHYLAMINO)PURINE

		Riboside from *Fusarium* sp.	Aglycone of *Fusarium* sp. riboside	Synthetic 6-hydroxy-(1-carboxyethylamino)-purine
Paper chromatography (R_F) *				
Solvent 1		0·73	0·66	0·67
Solvent 2		0·55	0·67	0·66
Solvent 3		0·57	0·59	0·60
Solvent 4			0·56	0·56
Solvent 5			0·41	0·41
Solvent 6			0·64	0·65
Solvent 7			0·15; 0·19	0·15; 0·19
Solvent 8			0·46	0·48
Paper electrophoresis (cm./hr. moved towards the anode at 25 V./cm.)				
*p*H 2·7 (phosphate–citrate)			−5·9	−5·6
*p*H 3·1 (phosphate–citrate)			+1·4	+1·6
*p*H 4·7 and 6·5 (acetate)		+9·5	+10·3	+10·3
Ultra-violet absorption at :				
*p*H 1,	maximum (mμ)	258	251, 278	250, 278
	minimum (mμ)	229	228, 270	227, 270
	inflexion (mμ)	274		
	*E*250 : *E*260	0·88	1·27	1·42
	*E*280 : *E*260	0·64	0·75	0·74
*p*H 6,	maximum (mμ)	255	248, 279	248, 279
	minimum (mμ)	227	226, 265	225, 266
	inflexion (mμ)	274		
	*E*250 : *E*260	1·03	1·44	1·45
	*E*280 : *E*260	0·67	0·97	0·85
*p*H 13,	maximum (mμ)	259	251, 278	250, 277
	minimum (mμ)	236	247, 261	246, 262
	inflexion (mμ)	266		
	*E*250 : *E*260	1·86	1·11	1·07
	*E*280 : *E*260	0·75	1·18	1·14

* With the exception of solvents 6 and 7, descending chromatography was used. Solvents were : No. 1, *iso*propanol–12·5 *M* hydrochloric acid–water (170 : 41 : 44, v/v/v); No. 2, *iso*butyric acid–1 *N* ammonia (100 : 60, v/v); No. 3, ethanol–1 *M* ammonium acetate, *p*H 3·8 (75 : 30, v/v); No. 4, as No. 3, but *p*H 7·5; No. 5, *n*-propanol–water (70 : 30, v/v); No. 6, 16 per cent ammonium bicarbonate; No. 7, ammonium sulphate–*n* propanol–0·1 *M* phosphate buffer, *p*H 6·8 (600 gm. : 20 ml. : 1,000 ml.); No. 8, *n*-propanol–16 *M* ammonia–water (60 : 30 : 10, v/v/v).

side isolated from the mould, a number of chromatograms were run with different solvent systems. A satisfactory separation was achieved in ammonium sulphate–*n*-propanol–0·1 *M* phosphate buffer, *p*H 6·8 (600 gm. : 20 ml. : 1,000 ml.). In this solvent the threonine derivative gave a single spot, whereas the α-alanine derivative divided into two spots, a behaviour also observed with its next higher homologue, that is, the α-aminobutyric acid derivative. The natural aglycone also gave two spots having the same R_F values as the synthetic α-alanine derivative.

In conclusion, the natural base and the synthetic compound prepared from α-alanine and 2-chloro-6-hydroxypurine appeared to be identical when compared by paper chromatography in eight solvent systems, by paper electrophoresis at four different *p*H values and by ultra-violet absorption spectra (Table 1). Furthermore, both gave guanine when heated with 4 *N* formic acid.

The evidence described above is probably the most that could reasonably be obtained with the minute amount of material available; on this basis it is proposed to assign provisionally the structure of 2-(1-carboxyethylamino)-6-hydroxy-9-D-ribofuranosylpurine to the new nucleoside isolated from *Fusarium*. The configurations of the amino-acid residue and of the ribose-base linkage are unknown, but it is probable that the latter is β-D by analogy with the majority of nucleosides and nucleotides found in Nature. The close similarity of the ultra-violet spectra of the nucleoside with those of guanosine[10] and of the riboside of 6-hydroxy-2-methylaminopurine[11] suggests that the ribose is linked to the 9 position of the purine base.

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Role of the Triplet State in the Photoreduction of Chlorophyll

IN 1948, Krasnovsky reported[1] that chlorophyll can be photochemically reduced, in dry pyridine, by ascorbic acid. The product is a pink pigment. The quantum yield of the reaction was not measured; but indirect evidence[2] indicates that it was of the order of magnitude of 10^{-2}. The reaction is reversible; the green colour of the solution returns slowly on standing, or more rapidly if a suitable oxidant is added. The regeneration of chlorophyll is never complete; part of it being converted into phæophytin. This and analogous photochemical reactions were studied extensively, in subsequent years[3], by Krasnovsky and Evstigneev *et al.* They have adopted the view that the reversible photoreduction of chlorophyll plays an essential part in photosynthesis and in chlorophyll-sensitized reactions *in vitro*.

The photochemical reduction must involve a direct reaction between an excited molecule of chlorophyll and a normal molecule of the reductant. Evstigneev *et al.*[4] demonstrated that ascorbic acid does not appreciably quench the fluorescence of chlorophyll, dissolved in pyridine. Since this eliminates the possibility that the first excited singlet state reacts directly with ascorbic acid, it has been postulated that the lowest triplet state undergoes this direct reaction. However, Fujimori and Livingston[5] were unable to detect any effect of ascorbic acid (at concentrations as high as 0·10 *M*) upon the rate of decay of the triplet state of chlorophyll, when the solvent was dry pyridine.

Recent work of Bannister[6] suggests a possible way of reconciling these apparently incompatible observations. He demonstrated that chlorophyll is not measurably reduced when it is illuminated in air-free, dry pyridine containing ascorbic acid. The reduction is readily detectable if the solvent contains 2 per cent, or more, of water. In pyridine containing 30 per cent of water and 0·10 *M* ascorbic acid, the quantum yield of photoreduction is about 0·10. Under these conditions, the pink pigment does not accumulate, and

oxidation does not restore chlorophyll but produces phæophytin. When the water content is 2 per cent, the quantum yield is several-fold lower, but the reaction is much more nearly reversible. These observations indicate that Krasnovsky's[1] supposedly dry pyridine contained an appreciable amount of water (possibly about 2 per cent).

We have measured the quenching of the triplet state of chlorophyll by ascorbic acid in pyridine, either dried with calcium hydride or containing a known concentration of water. All solutions contained 5×10^{-6} *M* chlorophyll *a* and were carefully deoxygenated. Confirming the published results of Fujimori and Livingston, we were unable to detect any quenching of the triplet in dry pyridine; however, in the presence of water, ascorbic acid unmistakably quenches the chlorophyll triplet. Our preliminary results are given in Table 1.

Table 1

k_1, sec.$^{-1}$ dry, no Q	k_1, sec.$^{-1}$ dry, Q added	k_1, sec.$^{-1}$ wet, Q added	$[Q]$ moles/l.	Water (per cent)	$k_Q \times 10^{-5}$ moles^{-1}/l./sec.$^{-1}$
850	750	3,400	0·0076	22·7	3·4
970	890	2,900	0·0017	22·6	10·0
780	780	2,450	0·0110	2·2	1·5

The rate of disappearance of the chlorophyll triplet conforms to the following equation[5,7], where $[GH]$, $[GH']$ and $[Q]$ represent the concentrations of ground-state chlorophyll, triplet-state chlorophyll and ascorbic acid, respectively, and $m = [GH] + [GH']$.

$$-\frac{d[GH']}{dt} = (k_1 + k_2[GH'] + k_3[GH] + k_Q[Q])[GH']$$

$$= (k_1 + k_2 m + k_Q[Q])[GH'] + (k_2 - k_3)[GH']^2$$

$$= k_I[GH'] + k_{II}[GH']^2$$

The averages of the numbers in the first two columns, 870 and 790, do not differ significantly, and what difference there is between them is in the wrong sense to be explicable in terms of quenching by ascorbic acid. The mean of the values of k_1, 840 sec.$^{-1}$, should be compared to the published values of k_1 and k_3 (ref. 7), as follows:

$$730 \text{ sec.}^{-1} + 2{\cdot}3 \times 10^7 \text{ sec.}^{-1} m^{-1} \times 5 \times 10^{-6} m = 850 \text{ sec.}^{-1}.$$

The agreement is fortuitously close. The quenching constants, k_Q, were obtained by dividing the difference between the values given in the first and third columns by $[Q]$. The spread of these preliminary values of k_Q is greater than would be predicted in terms of the reproducibility of the individual measurements. It suggests that, in the range investigated, k_Q is a function of the water content and possibly of the concentration of ascorbic acid. In spite of this uncertainty, the results establish the reality of the quenching by ascorbic acid in wet pyridine and show that it is about ten thousand-fold less efficient than the quenching by oxygen, quinone, etc.[5].

In the presence of water and ascorbic acid, flash illumination produces a long-lived form of chlorophyll in addition to the triplet state. Since its mean life is about 0·01 sec. and it absorbs more strongly at 4800 than at 5200 Å., it cannot be Krasnovsky's[1] pink pigment, but may be one of the 'electrode active' intermediates which were discovered by Evstigneev *et al.*[4].

The available evidence demonstrates that the photoreduction of chlorophyll cannot be an essential step in efficient, chlorophyll-sensitized photochemical reactions occurring in ordinary organic solvents. However, it is not justifiable to draw a similar conclusion about the photosynthesis of plants.

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PHYSIOLOGY

A Phenylthiocarbamide Test

425 Belgians (225 males and 200 females) were tested with phenylthiocarbamide and quinine following the technique of Harris and Kalmus[1,2]. Fisher's linear discriminant function was used to class the subjects as tasters and non-tasters. The chi square test for smoking habit gives no significant result, but the influence of sex and age is important as proved by a homogeneity test (Fisher 2×10 table for sex; $\chi^2 = 40{,}905$ with 5 d.f.) and a 'Student' test for the mean threshold for $\bar{T}$ and $\bar{t}$, males and females. ($t = 5{\cdot}04$ for $\bar{T}$ and $t = 3{\cdot}258$ for $\bar{t}$.) The correlation coefficient between age and phenylthiocarbamide is:

$r = -0{\cdot}390 \pm 0{\cdot}109$ for non-tasters

$r = +0{\cdot}032 \pm 0{\cdot}078$ for tasters

General results are summarized in Table 1.

Table 1

	Males	Females
n	225	200
Age	18–71 y.	14–26 y.
Number (t)	61	44
Frequency (t) (per cent)	27·11	22·00
Gene frequency (t)	0·521	0·469
Phenylthiocarbamide		
Mean total sample	7·498 ± 0·217	8·320 ± 0·247
Mean tasters	9·268 ± 0·107	10·045 ± 0·100
Mean non-tasters	2·738 ± 0·225	2·205 ± 0·180

Creteur obtained in 1952, with a different technique, 28·8 per cent of non-tasters among 679 subjects[3].

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Passage of Insulin through the Wall of the Gastro-intestinal Tract of the Infant Mouse

Mosinger, Placer and Koldovsky[1], in a recent communication, have shown that insulin administered orally to suckling rats 2 and 8 days old causes a large drop in blood-sugar levels, but does not do so in 21- and 30-day old rats. Previously I had found that the gut of the 8-day old mouse is permeable to insulin but that this permeability declines thereafter. This work was quoted by Halliday[2] and, although Mosinger *et al.* refer to that paper, they do not mention my findings.

LAB grey mice were used, the young being removed from the mother 2 hr. before the administration of insulin and kept in an incubator at 37° C. for the duration of the experiment. Administration was direct to the stomach with a polythene tube on a tuberculin syringe. Injections were made intraperitoneally. The insulin used was standard crystalline insulin (B.D.H., *A.* and *H.* 22 units/mgm.) dissolved in 0·9 per cent saline acidified with hydrochloric acid to *p*H 2·5. Controls were given an equivalent dose of acidified saline. Observation of convulsions and death did not provide a satisfactory method of measuring the passage of the intact insulin molecule through the gut wall, as infant mice survived hypoglycæmia far longer than did adults. This agrees with the findings of Himwich, Fazekas and Hamburger[3], who showed that in the rat the capacity of the infant brain to endure hypoglycæmia is associated with its lower cerebral metabolic requirement. Therefore, estimations of serum sugar were undertaken. Repeated bleedings of 8-day old mice necessitated micro-methods of sampling and estimation. Blood samples each of *c.* 5 µl. were taken from the tail into capillary tubes before administration of the hormone, and at 30 min. and 60 min. after. The tubes were sealed and stored at 4° C. until tested. The serum was separated by centrifugation and 2-µl. samples tested in duplicate by the method of Stern and Kirk[4]. An 'Agla' micrometer syringe was used for the titrations.

The results are shown in Fig. 1. It is evident that the majority show a significant fall in serum sugar at 7, 8 and 16 days, and that only a small minority show this at 22 days. At 8 days of age, doses of 1 unit/gm. may be effective. At 22 days even doses of 8–10 units/gm. usually have no effect. It is evident from these results that the gut of the suckling mouse at 8 days, and even at 16 days, is permeable to insulin administered orally in very large doses, and that this permeability has almost disappeared at 22 days. The disappearance of permeability to insulin appears to occur rather later and to be less clear-cut than that to antibodies in the suckling mouse. The doses necessary are enormous in comparison with those of the order of 0·001 unit/gm. which are effective by parenteral injection. The wide individual variation in response to the oral administration of insulin at all ages studied is surprising, and for this I have no explanation to offer.

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Seasonal Differences in Ovulating Hormone in the Merino Ewe

Cessation of cyclic ovarian activity in ewes during anœstrus is thought to be associated with changes in the level and/or type of gonadotrophic substances reaching the ovary[1]. This supposition cannot be tested by direct means at the present time, but a recently developed technique for synchronization of ovulation in ewes[2] suggests possibilities of an indirect examination.

Merino ewes were injected daily with 10 mgm. of progesterone in oil intramuscularly for 14 days in either April (the middle of the breeding season) or October (anœstrus). At the same time as the final progesterone injection each ewe received 500 I.U. of pregnant mare serum gonadotrophin subcutaneously. 48 hr. later groups of ewes were injected with either 300 I.U. of human chorionic gonadotrophin intra-

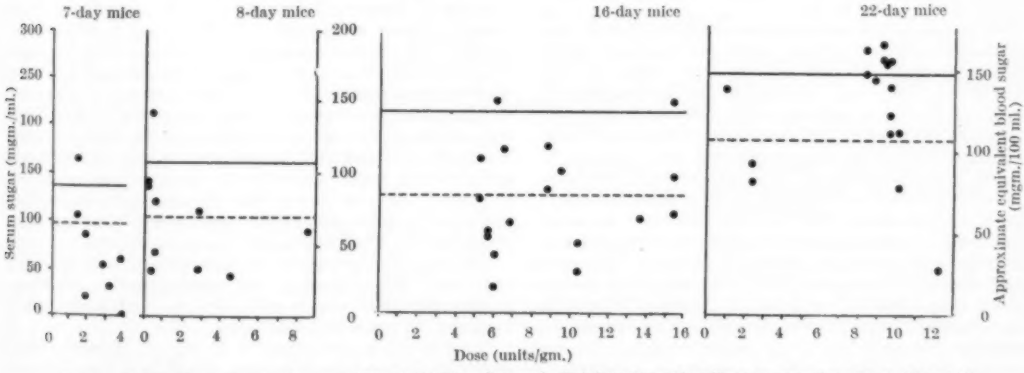

Fig. 1. Values of serum sugar in mgm./100 ml. 60 min. after oral administration of insulin to young mice of 7, 8, 16 and 22 days of age according to dose in units/gm. The corresponding approximate scales for blood sugar are inserted on the right for comparison with the serum sugar scale on the left; the blood sugar values being derived from the hæmatocrit values of 33 per cent for 8-day and 42 per cent for 16- and 22-day-old mice. The solid horizontal line represents the mean serum sugar-level before the administration of insulin and the broken line the value below which individual experimental results may be considered significant at the 5 per cent level

Table 1. Time of Ovulation in Merino Ewes

All ewes received 10 mgm. of progesterone intramuscularly daily for 14 days and 500 I.U. of pregnant mare serum gonadotrophin on the fourteenth day. The human chorionic gonadotrophin was given 48 hr. after the pregnant mare serum gonadotrophin

Season	Human chorionic gonadotrophin (I.U.)	No. of ewes	Ovulations occurring in following periods after human chorionic gonadotrophin					Mean time of ovulation (hr.)
			<22 hr.	22–29 hr.	29–44 hr.	> 44 hr.	Total	
April (breeding season)	300 intramuscular	4	3	3	0	0	6	23
	900 intramuscular	4	0	3	2	0	5	29
	300 intraperitoneal	5	0	1	6	0	7	32
October (anœstrus)	300 intramuscular	shorn 3	0	0	3	0	3	42
		unshorn 3	0	1	1	1	3	
	900 intramuscular	shorn 3	0	1	1	1	3	31
		unshorn 3	0	4	0	0	4	
	300 intraperitoneal	shorn 3	0	1	2	1	4	40
		unshorn 3	0	1	0	2	3	

Analyses of Variance—Estimated Times of Ovulation

Source of variation	DF		Mean square	
	April	October	April	October
Between doses				
300 intramuscular v. 300 intraperitoneal	1	1	261*	1
900 intramuscular v. rest	1	1	13	491†
Shearing effect		1		118
Interaction		2		19
Error	15	14	8·6	73·4

* $P < 0.001$. † $0.01 < P < 0.05$.

muscularly, or 900 I.U. intramuscularly, or 300 I.U. intraperitoneally. Laparotomy, which seems not to influence the time of ovulation[2], was performed 22, 29 and 44 hr. after the injection of human chorionic gonadotrophin. All ewes used in October were carrying eleven months wool but half were shorn 1–2 hr. prior to the injection of human chorionic gonadotrophin.

The treatment groups are shown in Table 1 together with the numbers of ovulations observed at the various times of laparotomy and the estimated mean time of ovulation for each group. The times of ovulation were estimated by visual examination. Ovulations which seemed to take place during the periods 19–32 hr. and 41–47 hr. were estimated to the nearest 1–2 hr.; others were estimated to the nearest 6–8 hr. The within-group variances were significantly greater in October ($\chi^2_{(1)} = 8{\cdot}78$, $0{\cdot}001 < P < 0{\cdot}01$) hence the two sets of data were analysed separately. A summary of the analyses is appended to Table 1.

The mean time of ovulation following 900 I.U. intramuscularly of human chorionic gonadotrophin was apparently constant at the two times of the year ($P = 0{\cdot}6$). A difference of approximately 20 hr. in the time of ovulation following 300 I.U. of human chorionic gonadotrophin intramuscularly suggests that during anœstrus there was present, at or soon after the time of injection, smaller amounts of a substance or combination of substances which acts in conjunction with human chorionic gonadotrophin to hasten ovulation in the breeding season. That the substance(s) is similar to human chorionic gonadotrophin is borne out by the response to the higher dosage-level, and since human chorionic gonadotrophin is generally thought to be similar to ovulating hormone[3] it is possible that there are seasonal differences in the endogenous production of ovulating hormone in Merino ewes. On this basis the greater variation in response in October suggests that individuals in this strain of Merino differ widely in the 'depth' of anœstrus.

In studying the hypothesis the following factors should be taken into account: (*a*) Pregnant mare serum gonadotrophin, given at the time of the final progesterone injection and known to be present in the blood of animals for several days, may influence the ovulatory response to human chorionic gonadotrophin. (*b*) Ovulation may be inhibited by high dosage-levels of human chorionic gonadotrophin[4]. (*c*) The rate of maturity of Graafian follicles after a constant progesterone – pregnant mare serum gonadotrophin treatment may be influenced by the time of year.

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Oxygen Consumption in Cattle in Relation to Rate of Increase in Environmental Temperature

In determining the responses of cattle to high, controlled temperatures, one of two procedures has usually been employed. Either the cattle were exposed for periods up to 7 hr. to temperatures ranging up to 110° F. (ref. 1), or they were exposed initially to lower temperatures which were increased progressively by small steps at 2-week intervals[2]. As there was some doubt as to whether cattle would react similarly to these two procedures, it was decided to subject the same animals to both types of heat exposure. It was hoped that this comparison would clarify any difference in the reactions of the animals produced by acclimatization or stress effects.

The specific objective of this experiment was to compare the changes in energy-levels (oxygen consumption) of cattle subjected to both acute and progressive types of heat stress.

Nine animals, including 3 Jersey, 3 Holstein and 3 Brown Swiss female calves, which had been raised at a constant temperature of 50° F., were available. At the beginning of the three treatments, shown by the three curves in Fig. 1, this group had reached 14 months of age. The results for all three breeds were

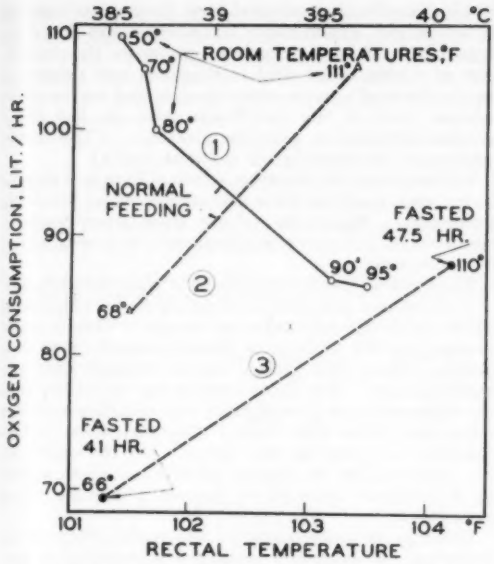

Fig. 1. Differences in the rates of oxygen consumption of cattle resulting from gradual and rapid changes in environmental temperature. Each plotted mean includes 18 observations for curves 1 and 3, and nine observations for curve 2

pooled since the changes with treatment were of primary interest. Oxygen consumption and pulmonary ventilation were measured with an open-circuit metabolism apparatus, which has been described in an earlier publication[2].

In the treatment which resulted in curve 1, the animals were kept for successive 2-week periods at 50°, 70°, 80° and 90° F., and for 4 days at 95° F. During this 2-month period of rising environmental temperature, the rectal temperature increased 2·1 deg. F. At the same time, the oxygen consumption decreased from 110 to 85 l./hr., even though the pulmonary ventilation increased from 49 to 129 l./min. and the body-weight increased from 347 to 365 kgm. Increased hydration may have been a factor in increasing the body-weight, as the plasma protein concentration decreased during this period[4]. The factors which tended to increase oxygen consumption, such as the greater muscular exertion required for high respiratory activity, the increased maintenance for greater body-weight, and the van't Hoff effect incident to high body-temperature, were obviously more than offset by decreases in thyroid activity[5] and consumption of total digestible nutrients (Johnson, H. D., and Ragsdale, A. C., unpublished work). The reduction in feed consumption at high temperatures was voluntary since feeding (except grain) was *ad libitum*.

Curve 2 for these same 9 animals shows that the effect of rapidly rising temperature on oxygen consumption was opposite to that of slowly rising temperature. Measurements made after the morning feeding at a room temperature of 68° F. were not as high as those made 5 hr. later after the room had been heated to 111° F. During the 5-hr. heating period, rectal temperature increased 1·9 deg. F., pulmonary ventilation increased from 45 to 140 l./min. and oxygen consumption increased from 83 to 107 l./hr. (significant at 0·05 level). The rise in oxygen consumption can be attributed to an increase in the muscular exertion of respiration, and the van't Hoff effect. The rapidity of heating the room and the cattle would tend to prevent a depression of any consequence by slow-acting factors. The available nutrients would remain relatively unchanged in a ruminant even though the appetite were depressed by the heat. It seems unlikely, also, that depression of thyroid activity was a major factor.

To eliminate effects of specific dynamic action related to time after feeding, the nine animals were fasted for 41 hr. at 65° F. temperature. Measurements were made at this time at a temperature of 66° F. and again 6½ hr. later after the room had been heated to 110° F. Averages of two such sets of fasting results are shown in curve 3. The 44 deg. F. rise in room temperature increased rectal temperature 2·9 deg. F. and pulmonary ventilation from 33 to 143 l./min. Oxygen consumption increased from 69 to 87 l./hr. (significant at 0·01 level). This rise in oxygen consumption was associated with increased respiratory activity and the van't Hoff effect. There is also the possibility that the combined stress effects of fasting and great heat may have elicited a particular group of reactions called by Selye[6] "the general adaptation syndrome". One of the main characteristics of this syndrome is hypersecretion of adrenal hormones including adrenaline, which is known to increase oxygen consumption in small animals.

Whatever mechanisms are involved, these results indicate that the reactions of cattle to high temperatures may differ with the rate of increase in environmental temperature. Gradual increases in temperature, within limits, depress oxygen consumption. Rapid increases in temperature elevate oxygen consumption.

This experiment was conducted in the Climatic Laboratory, Columbia, Missouri, as one part of a co-operative project between the University of Missouri and the Agricultural Research Service, United States Department of Agriculture.

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Release of Methionine labelled with Sulphur-35 from Muscle Tissue and Mitochondria

Continuous renewal of the protein molecule is effected by balance between the incorporation and the removal of amino-acids. From these two processes incorporation has been examined and some intermediate reactions are already known. So far as the second process, that is, intracellular degradation of proteins, is concerned, only basic experiments have been made. Simpson[1] and Steinberg and Vaughan[2] found, for example, that anaerobiosis and 2,4-dinitrophenole inhibit the release of labelled amino-acids from prelabelled liver slices. The

degradation of proteins was also found to be dependent on energy-yielding activity of the cell in homogeneous cultures of bacterial[3] as well as animal cells[4]. In subcellular particles, Korner and Tarver[5] found that anaerobiosis and 2,4-dinitrophenole inhibit the release of amino-acids from nuclei and mitochondria. The release from microsomes under the same conditions, however, is stimulated.

In the present communication the intracellular degradation of muscle proteins was studied. We have measured the release of methionine labelled with sulphur-35 from isolated rat diaphragm and from muscle mitochondria. 24 hr. before the experiment the rat received 150 μc. of ^{35}S-methionine per 100 gm. body-weight by intraperitoneal injection. Trichloracetic acid-soluble ^{35}S-methionine in one-half of the diaphragm and in a sample of mitochondria served as initial values. The remaining parts were incubated. The increase of free labelled methionine after 3 hr. incubation of the diaphragm and 2 hr. incubation of mitochondria under usual conditions was determined. This amount was then expressed in percentage protein-bound ^{35}S-methionine.

The mitochondria or homogenated diaphragm were extracted with trichloracetic acid in final concentration of 5 per cent. Following acid hydrolysis, labelled methionine of the protein precipitate was separated from cystine and cysteine by their precipitation as mercaptides. The amino-acids in neutralized trichloracetic acid extract of tissue (or mitochondria) and medium were concentrated on 'Dowex-50'. Taurine and sulphate pass through. Amino-acids were then eluted, and, after acid hydrolysis, cystine, cysteine and homocysteine were precipitated as mercaptides. Methionine of protein as well as of the trichloracetic acid extract was oxidized to sulphate and radioactivity was measured in benzidine sulphate precipitate with an accuracy of 2·5 per cent.

The results given in Table 1 demonstrate that the amount of labelled methionine released from both diaphragm and mitochondria is higher in a medium with 2·4-dinitrophenole and under anaerobic conditions. (The activity of proteolytic enzymes measured at *p*H 3·6 and 9·0 is not changed under these conditions.) On the contrary, the amount of labelled methionine released from liver mitochondria is, as in the experiments of Korner and Tarver[5], depressed. (Protein degradation, with the exception of release of labelled methionine, was measured on the basis of non-protein nitrogen and amino-acids increase and in the diaphragm also on the basis of non-collagenous proteins decrease. The same results are obtained by all these methods.)

Furthermore, a different reaction of liver and muscle tissue was observed after the action of amino-acids antagonist. While the release from liver slices is inhibited by *p*-fluoro-phenylalanine[1,2] it is stimulated from diaphragm.

The present results demonstrate that the degradation of muscle proteins is enhanced under conditions which limit the utilization of energy. This is quite contrary to the findings in tissues studied up to now (namely, liver and kidney slices, reticulocytes and macrophages). For these cells it was assumed that the degradation of protein involves a complex acceptor substance rather than water. The formation of this complex is dependent on energy. On the contrary, the degradation of muscle proteins is given rather by hydrolytic splitting of peptide bonds by tissue cathepsins.

What is the mechanism by which the effect of the inhibitors of oxidative processes is realized in our experiments? If it is not by influencing the activity of tissue cathepsins, they must affect some other reaction which then makes proteins more accessible to the hydrolytic action of cathepsins. It is important to note that not only inhibitors of oxidative processes but also antagonists of amino-acids stimulate the release of labelled methionine from isolated diaphragm. It is possible that the intracellular degradation of muscle proteins is always stimulated under conditions of inhibition of proteosynthesis.

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Table 1. Release of Methionine labelled with Sulphur-35 from Rat Diaphragm and Mitochondria under Different Conditions of Incubation

Conditions	Diaphragm	
	c.p.m. released (per 1 gm. wet wt.)	Percentage of ^{35}S - methionine released*
Normal†	460 ± 10	1·40 ± 0·05
Anaerobiosis	1,110 ± 20	3·64 ± 0·44
2, 4-Dinitrophenole (10^{-4} *M*)	1,190 ± 50	—
p-FPA (10^{-3} *M*.)‡	1,290 ± 80	4·14 ± 1·04

	Mitochondria*		
	Rat Skeletal muscle	Pigeon Breast muscle	Rat liver
Normal§	0·61	1·09	5·28
Anaerobiosis	2·20	3·18	4·43

* $\frac{\text{c.p.m. of released } ^{35}\text{S-methionine}}{\text{c.p.m. of protein-bound } ^{35}\text{S-methionine}} \times 100$.

† Krebs-Ringer bicarbonate buffer containing 200 mgm. per cent glucose and carrier methionine in conc. 0·12 mgm./ml. Gas phase was 95 per cent oxygen and 5 per cent carbon dioxide.

‡ *p*-Fluoro-phenylalanine.

§ 3 ml. of medium contained: 50 μ*M* potassium succinate, 20 μ*M* adenosine triphosphate, 0·01 *M* potassium phosphate buffer of *p*H 7·4, 0·005 *M* magnesium chloride, 0·003 *M* ethylenediamine tetraacetic acid and 1 ml. of mitochondria suspension in 0·25 *M* sucrose. Isotonicity was maintained by adding potassium chloride. Concentration of carrier methionine was 0·12 mgm./ml. Gas phase was oxygen.

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Duration of Stimulation of Phagocytic Activity of the Reticulo-Endothelial System after Cessation of Treatment with Diethylstilbœstrol

Nicol *et al.* have reported previously that diethylstilbœstrol is a strong stimulant of the phagocytic activity of the reticulo-endothelial system[1]. The present research was designed to ascertain the period of time during which the stimulative effect would persist after cessation of treatment with diethylstilbœstrol.

The following experiments were carried out on 80 male white mice (*T.O.* Swiss strain) of 20–28 gm. body-weight. Fifty-five animals were each given one subcutaneous dose of 0·5 mgm. diethylstilbœstrol in 0·05 ml. arachis oil daily for 6 days. On the second day after the treatment with diethylstilbœstrol ceased, the phagocytic activity of the reticulo-endothelial system was measured in five animals by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood[2],

the procedure used being that described previously[1], and the total body phagocytic activity or phagocytic index being denoted by the symbol K. On each successive day thereafter, up to the twelfth day, the phagocytic activity was measured in further groups of five animals.

Twenty-five of the animals were used as controls and were given 0·05 ml. arachis oil once daily for 6 days; the phagocytic activity was then measured on the eighth day by the carbon method referred to above. The arachis oil controls showed a phagocytic index or K value of 18 ± 5·8.

Table 1. Duration of Stimulation of Phagocytic Activity of the Reticulo-Endothelial System after Cessation of Treatment with Diethylstilbœstrol

Day after cessation of 6-day treatment with diethylstilbœstrol	Phagocytic index (K value)	Percentage change in body-weight
2nd	90 ± 10·8	−5
3rd	102 ± 13·1	−7
4th	56 ± 9·8	−3
5th	62 ± 11·5	+2
6th	55 ± 6·3	+5
7th	36 ± 4·7	+5
8th	34 ± 1·2	+10
9th	23 ± 1·4	+5
10th	24 ± 1·5	+10
11th	24 ± 2·7	+10
12th	22 ± 4·1	+9
Control values in 25 animals	18 ± 5·8	

The results are shown in Table 1. Compared with the controls it can be seen that after treatment with diethylstilbœstrol ceased the stimulative effect on the phagocytes persists for approximately one week, during which time it gradually diminishes. By the eighth–ninth day the phagocytic index or K value has returned to within normal control limits.

A corresponding change in the body-weight of the animals also occurs. It was reported previously[3] that when the daily dose of diethylstilbœstrol rises above 0·1 mgm. the animals lose weight. From Table 1 it can be seen that this loss in weight continues for about 4 days after the diethylstilbœstrol treatment has ceased and thereafter there is gain in body-weight.

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HÆMATOLOGY

Observations on Globin, Hæmoglobin and Haptoglobin using a Rabbit Antiglobin Serum

An antiserum against human globin was raised in a rabbit by immunization with globin prepared from adult hæmoglobin by the methods of Anson and Mirsky[1] and Jope, Jope and O'Brien[2]. A 1 per cent solution was injected subcutaneously in Freund's adjuvant[3], and intravenously. The resulting antiserum precipitated both human globin and human hæmoglobin; 1 ml. precipitated about 0·5 mgm. of hæmoglobin.

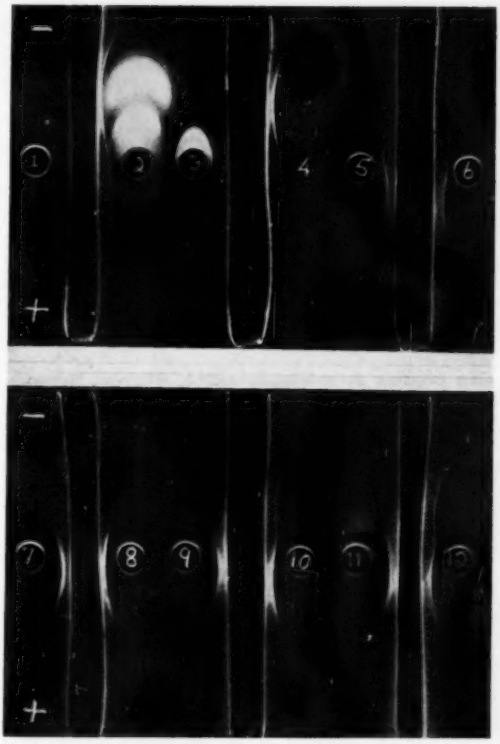

Fig. 1. Immunoelectrophoresis with antiglobin antiserum

A series of immuno-electrophoretic analyses in *p*H 7·0 0·05 M phosphate buffer in 0·8 per cent agar, using this antiserum, is shown in Fig. 1. The antigen cups are numbered. No. 1 contains human serum, which shows a faint precipitin line of a protein which has moved slightly towards the anode. This probably is haptoglobin-bound hæmoglobin. A 5 mgm./ml. solution of globin in 0·9 per cent sodium chloride was placed in cup 2. This shows a precipitin line due to protein which has moved towards the cathode, and in addition, a heavy precipitate of protein in the agar. A 1 mgm./ml. solution of globin in cup 3 shows a similar precipitate in the agar, but no immunological precipitate has occurred, presumably because of complete precipitation of the globin before it reached the antibody. The same concentration of hæmoglobin in cup 4 shows a precipitin line of similar electrophoretic mobility to the globin in cup 2.

The remaining analyses illustrate the effect of increasing the concentration of either globin or hæmoglobin in human serum. The same series of concentrations of both proteins was used, that is, 0·3, 0·6, 0·9 and 1·2 mgm. per 1 ml. of serum. The mixtures containing globin in cups, 5, 7, 9 and 11 and hæmoglobin in cups 6, 8, 10 and 12 are in order of increasing concentration.

All the hæmoglobin mixtures show a curved precipitin line of protein which has moved slightly towards the anode. In addition, at 10 and 12, there is a second arc of precipitation on the cathode side of the origin, the two arcs of precipitate forming a continuous line. Filter-paper electrophoresis of the mixtures in cups 6 and 8 showed the hæmoglobin

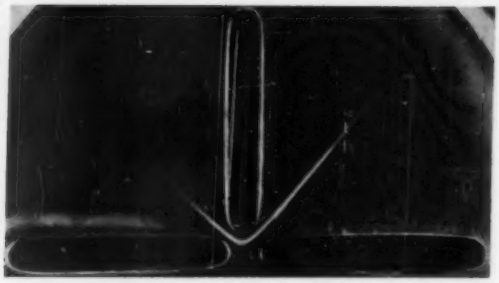

Fig. 2. Gel diffusion plate. Antiglobin antiserum in the vertical trough. Hæmoglobin in serum in the left-hand trough. Hæmoglobin in saline in the right-hand trough

to be entirely haptoglobin-bound, whereas those in cups 10 and 12 showed both haptoglobin-bound and also free hæmoglobin. The precipitin line on the anode side of the origin is therefore interpreted as indicating haptoglobin-bound hæmoglobin, that on the cathode side being free hæmoglobin, similar in mobility to that in cup 4. Similar double arcs of precipitate have been described by Boivin and Hartmann[4].

All the globin mixtures show an arc of precipitate of identical mobility to the haptoglobin-bound hæmoglobin, strongly suggesting that haptoglobin also binds globin. In addition, the higher concentrations of globin at 9 and 11 show precipitation on the cathode side of the origin similar to that shown by free globin in cups 2 and 3. If it is assumed that this precipitate is free globin, this would indicate that the globin-binding capacity of human serum haptoglobin is similar to its hæmoglobin-binding capacity.

Allison and Humphrey[5] deduced that when antigen and antibody at optimal proportions diffuse towards each other from troughs at right angles to each other in an agar gel, a straight line of precipitation is produced. The angle θ between the line and the antigen trough is related to the diffusion coefficients of the antigen (Dg) and antibody (Db) by the formula: $\tan\ \theta = (Dg/Db)^{1/2}$. They substantiated this by measurements of such angles produced by a series of antigens covering a wide range of molecular size, and their equivalent antisera. The antiserum against human globin enables us to measure such angles for free hæmoglobin, and for haptoglobin-bound hæmoglobin, that is for two proteins, antigenically identical, but of considerably different molecular size.

Fig. 2 is a photograph of such an agar plate, in which the antiserum was placed in the vertical trough, and hæmoglobin in identical concentrations, at optimal proportions to the antiserum, in the horizontal troughs. The hæmoglobin solution in the left-hand trough contained human serum at a concentration equivalent to 1 ml. per 0·5 mgm. of hæmoglobin. Filter-paper electrophoresis confirmed that all the hæmoglobin in this preparation was haptoglobin-bound. Straight lines of precipitation are obtained giving values of θ for free hæmoglobin of 51°, and for haptoglobin-bound hæmoglobin of 43°.

Assuming a diffusion coefficient of rabbit γ-globulin of $3{\cdot}8 \times 10^{-7}$ cm.^{2} sec.$^{-1}$, the diffusion coefficient of free hæmoglobin can be calculated as $5{\cdot}8 \times 10^{-7}$ (Gutter, Sober and Peterson[6] reported a value of $6{\cdot}0 \times 10^{-7}$ for carboxy-hæmoglobin). The diffusion coefficient of the complex is calculated as $3{\cdot}3 \times 10^{-7}$.

These results show that straight lines can be produced in this system when an antibody is treated at optimal proportions with its antigen at two widely different molecular sizes. The difference of molecular sizes is indicated by differences in the values of the angles θ.

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Frequencies of the Haptoglobin Serum Groups among Blood Donors from Austria and Germany

The frequency of the gene Hp^1 among the European populations so far tested have shown only a slight deviation of 40 per cent (Table 1). Samples from Austria and Germany have hitherto not been examined. For our studies we used sera of 201 blood donors from Münster, Westphalia (Germany), 273 blood donors from Bavaria (three districts of Upper Bavaria)[1], and sera of 621 blood donors from Vienna and environs (Austria).

Table 1

Author(s) and ref.	Population	*n*	Hp^1
Galatius-Jensen[7]	Denmark	2,046	0·397
Tarukoski[8]	Sweden	1,003	0·376
Nyman[9]	Sweden	275	0·451
Mäkelä[10]	Finland	891	0·326
Fleischer *et al.*[11]	Norway	1,000	0·363
Allison *et al.*[12]	Gt. Britain	218	0·406
Moullec *et al.*[13]	France	406	0·402
Allison *et al.*[12]	Basque	107	0·379
Harris *et al.*[14]	Italy	422	0·380
Bütler *et al.*[15]	Switzerland	920	0·396

A modified technique of starch-gel electrophoresis[2,3] has been applied using polyvinyl chloride trays with 10 channels (internal dimensions: 25 cm. long, 1 cm. wide, 0·8 cm. deep) for electrophoresis. The hydrolysed starch was obtained by the Connaught Medical Research Laboratories, Toronto, prepared by Smithies' method. Three serum samples to which hæmoglobin was added amounting to 300 mgm. per cent were placed in each channel at a distance of about 7 cm. The sera were applied to filter paper, Schleicher and Schüll No. 5703. A borate buffer (*p*H 8·4) was used for the gel as for the electrode chambers. Electrophoresis was performed with the high-voltage apparatus described by Wieland and Pfleiderer[4]. The voltage gradient was kept at 9 V./cm. and 95 m.amp. for 20 channels during a period of 3½ hr. After electrophoresis the slides were stained with a benzidine or *o*-dianisidine reagent for peroxidase activity.

The frequency of the three haptoglobin types and the gene frequency of Hp^1 are shown in Table 2. No individual of the *Hp* 0–0 group has been detected. The frequencies of the three phenotypes are in good agreement with the genetical theory of Smithies and

Table 2

	n	*Hp* 1–1	*Hp* 2–1	*Hp* 2–2	Hp^1	χ^2	*P*
Bavaria	273	19·4	54·2	26·4	0·465	2·188	>10 cent
	obs.	53	148	72			
	exp.	59·1	135·8	78·1			
Münster	201	19·9	46·8	33·3	0·433	0·453	>50 per cent
	obs.	40	94	67			
	exp.	37·6	98·7	64·7			
Vienna and environs	621	16·1	48·2	35·7	0·402	0·002	>95 per cent
	obs.	100	299	222			
	exp.	100·2	298·5	222·4			
Total	1095						

Walker[5,6] (χ^2-values between 10 and 50 per cent). The differences between the three samples are not significant ($\chi^2 = 6{\cdot}46$; $d.f. = 2$; P: 5 per cent $> P > 2$ per cent). The frequency of the Hp^1 in the German samples is somewhat higher than in most other European populations that have so far been tested.

The tests were carried out at the University Institute of Anthropology and Human Genetics at Munich (Director: Prof. K. Saller) under a grant given by the Deutsche Forschungsgemeinschaft.

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Fœtal Hæmoglobin in the Monkey

Some well-known physico-chemical differences between human adult and fœtal hæmoglobin prompted us to find if similar differences exist between hæmoglobins from adult and new-born monkeys. The present communication describes the examination of the blood of 8 adult and 4 new-born monkeys (*Macacus rhesus* of North India) by the alkali resistance test and paper electrophoresis. Pregnant monkeys were obtained and were delivered in the cage to provide the new-born monkeys. Heart blood from the new-born and venous blood from the adult monkeys was collected in citrate solution. The alkali denaturation method of Singer *et al.*[1] was employed for determining the proportion of alkali-resistant hæmoglobin. Electrophoresis was carried out in 'Elphor' apparatus using paper strip No. 2043 *a* Mg 1 at 250 V. for a period of 16 hr. in veronal buffer of *p*H 8·6 and ionic strength 0·05.

Fig. 1. Electrophoretic mobilities of hæmoglobin from adult and new-born monkeys

Fig. 1 shows the electrophoretic mobilities of hæmoglobin from adult and new-born monkeys. Hæmoglobins from both the adult and the new-born monkeys consist of one component only and have identical electrophoretic mobilities. Jacob and Tappen[2], however, obtained more than one component in a few of the other species of adult monkeys examined by them.

Table 1 shows the percentage of alkali-resistant hæmoglobin found by us. Adult monkeys contain 1·0–5·0 per cent alkali-resistant hæmoglobin, with an average of 2·9 per cent, whereas new-born monkeys contain 38·8–60·0 per cent, with an average of 48·9 per cent. Though the number of animals in the new-born group is rather small, the results are sufficiently indicative of the existence of a specific fœtal hæmoglobin characterized by resistance to alkali denaturation. In a recent communication, Beaven and Gratzer[3] have obtained evidence of a specific fœtal hæmoglobin by electrophoresis in agar gel. By using a moving-plate spectrograph, these workers have further shown that fœtal rhesus monkey hæmoglobin has the tryptophan fine-structure band at about 289·7 mμ, whereas the adult pigment has its band at 291·0 mμ—a condition very similar to that for fœtal and adult human hæmoglobins.

Characterization of the fœtal monkey hæmoglobin by its resistance to alkali denaturation, however, allows its easy detection by a relatively simple procedure. The quantities of fœtal hæmoglobin in the new-born monkeys have been measured at intervals of 15 days for 90 days in order to ascertain the time of disappearance of the fœtal pigment. Table 2 shows the percentage of fœtal hæmoglobin in the blood of these young animals. It was found that the amount of fœtal hæmoglobin is reduced to 1·4–

Table 1. Percentage of Alkali-resistant Hæmoglobin in Adult and New-born Monkeys

Animal No.	Adult Alkali-resistant hæmoglobin (per cent)	Animal No.	New-born Alkali-resistant hæmoglobin (per cent)
1	1·8	1	38·8
2	5·0	2	50·0
3	4·5	3	47·0
4	2·5	4	60·0
5	3·4	—	—
6	1·0	—	—
7	3·0	—	—
8	2·0	—	—
Mean	2·9	Mean	48·9
Range	1·0–5·0	Range	38·8–60·0

Table 2. Percentage of Alkali-resistant Hæmoglobin of Monkeys at Different Days after Birth

Days	No. 1	No. 2	No. 3	No. 4*
0†	38·8	50·0	47·0	60·0
15	35·5	46·0	42·0	50·0
30	15·5	25·8	20·8	19·8
45	8·4	20·0	6·8	—
60	5·0	18·2	1·5	—
75	2·5	9·2	1·5	—
90	2·4	6·2	1·4	—

* Animal No. 4 died on the fortieth day.
† 0 day indicates just after birth.

6·2 per cent in the course of 60–90 days, with a variable rate of disappearance. For human new-born babies, though the amount of fœtal hæmoglobin varies between 30·0 and 85·0 per cent at birth[4], it gradually disappears, so that not more than 10·0 per cent is found after 4 months[5] and less than 1·0 per cent after the first few years of life[6].

The close similarity between monkey and human hæmoglobin reported here and elsewhere[3] indicates the need for further work using various parameters that characterize different hæmoglobin types.

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Differences in Numbers of Erythrocytes between Inbred Lines of Chickens

No breed differences in numbers of red blood cells have been reported in poultry[1] although Lucas and Dennington[2] have shown small differences in hæmoglobin concentration and in the volume of packed red cells between inbred lines of the same breed (White Leghorns, as used in this work). In the results reported here, much more extensive differences in all three characters, which appear to represent a distinct form of variation, have been found.

Adult females of the Reseaheath inbred White Leghorn strains *C*, *I* and *W* were used[3]. All three lines have been brother–sister mated continuously for over 20 generations, but the attained level of inbreeding is probably well below the calculated figure, as segregation of genes for differences in red cell antigens is still occurring[4].

Egg production in the birds tested varied somewhat, but no differences could be detected between birds in lay and those out of production. The method of housing, too, differed in that some of the *C*-line and all the *I*-line birds (the blood from these birds was kindly supplied by Dr. D. G. Gilmour, School of Agriculture, Cambridge) were kept in semi-intensive pens, whereas the rest were housed in battery cages. Again, there were no differences due to method of housing.

The birds were bled from the brachial vein into heparin, approximately 2 ml. being removed for determining hæmoglobin content and packed cell volume. Red blood cell counts could be carried out only on birds of the *C* and *W* lines.

For the hæmoglobin estimation, the concentration of oxyhæmoglobin was read in a Spekker photodensitometer, human blood with a known concentration being employed as the standard. Packed cell volume was determined by the microhæmatocrit method, using a micro centrifuge at 12,000 r.p.m. for 5 min. For red cells counts the methyl violet stain of Natt and Herrick[5] was employed and counting carried out visually with the improved Neubauer hæmocytometer.

The results in Table 1 suggest that the differences in red cell characteristics between the inbred line *C* and the others are due to a reduction in the number of red blood cells in the *C* line. Determination of plasma volumes with the dye dilution technique using azovan blue showed no substantial line differences, and indicates that a real reduction in numbers of circulating red cells is involved.

Table 1. Differences in Red Cell Characters between Inbred Lines

Line	*W*	*I*	*C*	6 and 15 (ref. 2)
Hæmoglobin concentration (gm./100 ml. blood)	10·3	9·9	7·2	9·86
	0·35 N.S.		0·35*	
S.E. of difference		0·16*		
Number of birds	39	10	39	155
Hæmatocrit (packed cell volume, per cent)	33·6	32·6	23·8	30·9
	1·12 N.S.		1·12*	
S.E. of difference		0·77*		
Number of birds	31	10	33	155
Number of red blood cells (millions/ml.)	3·06		2·30	3·03
S.E. of difference		0·09*		
Number of birds	11		12	155
Mean cell volume (μc.)	109·8		104·0	102·0
Mean cellular hæmoglobin (μμgm.)	33·7		31·4	33·1
Mean cellular hæmoglobin concentration (per cent)	30·7	30·3	30·3	32·3

* Significant at the 0·01 per cent level. N.S., not significant.

Examination of blood films failed to reveal any evidence of reticulocytosis or other cytological signs of anæmia in the *C*-line birds. The heart-rate (as determined with a Cossor electrocardiograph) of birds of the *C* line averages 333, a value not significantly different from that of 338 for the *W* line. Their mature body-weight (1·57 kgm.) is well below that of the *W* line (2·00 kgm.), but exceeds that of the *I* line (1·29), whereas egg production (over a 44-week period) of the *C* line is 134 eggs, compared with only 80 for *W*, and 86 for the *I* line.

Tests so far carried out show no relationship between the observed oligocythæmia in line *C* with any hæmoglobin abnormalities. Electrophoresis of hæmoglobin solutions from birds of the *C* and *W* lines reveal no differences in the concentrations or mobilities of the two components previously reported by Johnson and Dunlap[6] either on filter paper at *p*H 8·6 or on agar gel at *p*H 6·4.

Further examination of the hæmoglobins for differences in resistance to alkali denaturation proved equally negative. Evidently a situation comparable with the human hæmoglobinopathies is not responsible for the differences observed in our inbred lines of poultry. Differences were, however, observed in resistance of the erythrocytes to osmotic pressure. Cells from birds of line *C* begin to hæmolyse at

Table 2. Osmotic Resistance of Red Blood Cells

Line	Beginning of hæmolysis (sodium chloride, per cent)	End of hæmolysis (sodium chloride, per cent)
C	0·495–0·480	0·300–0·285
W	0·435–0·420	0·300–0·285
Values quoted by Sturkie (ref. 7)	between 0·47 and 0·40	0·28

higher concentrations of saline than do those of the *W* line.

Figures based on visual assessments of the beginning and end of hæmolysis are given in Table 2. The proportions of hæmoglobin in solution at different concentrations of saline were also estimated using a 'Unicam' spectrophotometer set at 540 mμ. Expressed as a percentage of the amount released by distilled water, these are shown in Fig. 1.

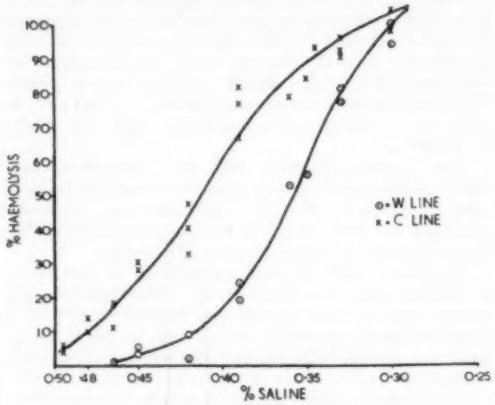

Fig. 1. Osmotic fragility curves

Diminished osmotic resistance is frequently indicative of lowered resistance to mechanical damage, leading to a reduction in the average life-span of the cells. This possibility is now under investigation as is the mode of inheritance of the condition.

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PATHOLOGY

Alkaline Phosphatase in the Meninges and in Meningiomas

Little is known about the factors which influence cartilaginous and bony metaplasia in tumours apart from the presence of calcified debris and mucinous infiltration in the stroma of these tumours. Willis[1] discusses the association of frequent bony metaplasia in carcinomas of the large intestine, and suggests that this may be associated with the alkaline phosphatase content of these tumours. He furthermore suggests that studies of phosphatases and other enzymes should be undertaken in tumours which show a tendency to bony metaplasia. The presence of an alkaline phosphatase in the 'fibroblasts' of fibrous dysplasia[2,3] and in the so-called fibrocytes of ossifying fibromas[3] certainly lends support to the ossifying properties of this enzyme in certain tumours.

In view of the frequency of metaplastic bone formation in the dura mater, especially in the region of the falx cerebri and the high incidence of calcification and ossification in certain types of meningiomas, it was decided to investigate this problem with regard to its possible association with alkaline phosphatase.

For this purpose two meningiomas were investigated histochemically. Morphologically one was classified as a meningothelial and the other as a psammomatous type of meningioma. In addition, segments of the arachnoid and dura mater in the vicinity of the superior sagittal sinus were obtained from three different autopsies shortly after death and examined histochemically.

Alkaline phosphatase was demonstrated according to the methods described by Pearse[4]. A strong alkaline phosphatase was observed in the arachnoidal cap cells of the arachnoidal granulations and arachnoid membrane, and also in the arachnoidal cell nests in the substance of the dura and arachnoidal membranes. In the meningothelial meningioma a strong enzyme reaction was elicited by all the tumour cells and in the psammomatous type, the enzyme was localized to the cells around the psammoma bodies.

The finding of an alkaline phosphatase in the tumours and their cells of origin may offer some explanation for the frequency of metaplastic ossification in these sites. Of more importance, however, is that it supplies the pathologist with a method by which the relative proportions of arachnoidal cells and fibroblasts can be readily assessed in the tumours.

Details concerning these findings and further experimental work will be published elsewhere. This work was supported by a grant from the Council for Scientific and Industrial Research.

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Isolation of a Cytopathogenic Agent from Skin Lesions of Cattle

Recently, we observed an outbreak of a disease in indigenous cattle at Gikongoro, Ruanda-Urundi, characterized by lesions on the teats and occasionally on other parts of the body and on the buccal mucosa. Material was obtained from nine cases, and with each sample transmission experiments were carried out in cattle. Following intradermal inoculation or scarification of the mucous membrane of the tongue, a definite reaction was noticed in five out of nine animals. This reaction was characterized by the development of necrotic lesions at the inoculation

site, which showed some resemblance to those described in both natural and experimental cases of lumpy skin disease[1–3].

An excised nodule from an experimentally infected calf was used for the inoculation of monolayers of bovine testis cells. Some cytopathic changes were observed already at the first passage, but they were much more marked at the second passage, and consisted of intranuclear inclusions and the formation of large syncytia. The syncytia covered the whole monolayer in a few days and led to complete destruction of the cell sheet. Similar changes were observed in cultures of lamb testis cells. The time of appearance and the evolution depended upon the cell type and the age of the monolayer at the time of inoculation.

The tissue culture-passaged virus was inoculated intradermally into a one-month-old calf, which developed a necrotic lesion about 5 cm. in diameter at the site of inoculation. Rabbits and guinea pigs, inoculated by scarification of the skin with the tissue culture-passaged virus, showed a marked local reaction after a few days.

The agent we isolated shows characteristics somewhat intermediary between those of the Neethling and Allerton types of viruses recovered from cases of lumpy skin disease by Alexander *et al.*[2] in South Africa and by Prydie and Coackley[4] in Kenya. It produces cytopathic changes closely resembling those produced by the Allerton type, but the reactions observed in experimentally infected bovines are similar to those induced by the Neethling type. On the other hand, the clinical symptoms in the natural cases are milder and somewhat different from those commonly described in other African countries.

Further research is in progress and a detailed report of the experiments will be published elsewhere.

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Induction of a Chorion-epitheliomatous Tumour in the Rat

Chorion-epithelioma of the uterus is a relatively rare tumour with many interesting features. It occurs only during or after pregnancy, and invasion and metastasis only take place *post-partum* or *post-abortum*. Spontaneous regressions occur in a small but significant number of cases[1]. It is the only known instance of naturally occurring tumour homograft[2]. A response to œstrogen therapy has been recorded[3] and remissions with folic acid antagonists have been reported[4].

The experimental induction of this tumour in laboratory animals would be of great interest. To our knowledge there are no records in the literature of spontaneous or induced chorion-epitheliomas in rodents, though Fischer and Kuehl[5] mention chorion-epitheliomatous structures in the ovaries of guinea pigs.

A tumour of the uterus in the pregnant rat has been induced in the following manner. A rat fœtus was removed after 2 weeks gestation by Cæsarean section, the placenta being left *in situ*. Benzpyrene powder was dusted into the gestational sac and the uterus then closed. After 4 months, vaginal bleeding was noted and exploratory laparotomy revealed the presence of enlarged cystic ovaries and thickened uterine horns containing hæmorrhagic fluid. Five months after the application of benzpyrene, a large tumour about 40 mm. × 30 mm. was palpated in the abdomen and the animal was killed. At autopsy a large, white, firm, nodular tumour measuring 60 mm. was seen in the uterine horn. There were numerous secondary deposits in the abdominal cavity. In the lungs there were pneumonic patches but no secondary deposits. Histologically, the tumour was a highly anaplastic growth having both sarcomatous and decidual elements. Many mitoses were present. This tumour was transplantable with a 100 per cent yield on subcutaneous passage and kills the host in 7–10 days.

The Ascheim–Zondek test was negative—but it was equally negative with urine from normal pregnant rats. Animals bearing intrauterine transplant tumours or very large subcutaneous growths have hæmorrhagic or lutein cysts in the ovary.

The action of folic acid antagonists on this tumour is being studied. Preliminary investigations have shown that it is sensitive to 'Methotrexate'[6] if the drug is given in high doses. An attempt is also being made to determine the sex of the tumour cells.

Twenty-five rats were similarly treated with benzpyrene and it appears that tumours are developing in two more animals.

A detailed report on this work will follow shortly.

I am indebted to Dr. S. S. Epstein for helpful discussion and criticism, and to Dr. C. P. Cherry for histological examinations.

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Presence of Chicken Tumour Virus in the Sarcoma of the Adult Rat inoculated after Birth with Rous Sarcoma Tissue

The heterotransplantation of Rous sarcoma to newborn rats results in lethal hæmorrhagic disease occurring in these animals[1–3]. Svet-Moldavsky[3] succeeded in determining the infectious virus in hæmorrhagic cysts, while Zilber and Kryukova[1] and Kryukova[4] had negative results. In our experiments, the virus

was not found in every case, but we observed it in some of the animals[3,5]. Svet-Moldavsky[6] has also shown that Rous virus can induce the formation of rat sarcomas which do not contain the virus. On the basis of our material we came to a similar conclusion. In 2 out of 87 young rats inoculated 1–7 days after birth with a single dose of 0·3 ml. of cut fresh Rous sarcoma tissue diluted with physiological saline to make a 50 per cent suspension, large sarcomas were found in thigh muscle tissue in addition to hæmorrhagic cysts occurring in other parts of the body. The first case was observed 5 months and the second one 7 months following inoculation of Rous sarcoma; the cyst was localized in the axillary region, whereas the sarcoma grew in the thigh muscle of the hind leg.

Table 1. Tests for the Presence of Avian Tumour Virus in the Wall of the Hæmorrhagic Cyst, in Blood, Sarcoma and its First Passage, made on 20–29-day old Chickens

Material taken from the adult rat inoculated after birth with Rous sarcoma tissue

Inocula	Dose of 50 per cent tissue suspension in physiological saline (i.m.)	No. of chickens	No. of chickens dead from tumours
Cyst wall	0·3 ml.	3	1
Blood from the cyst	2·5 ml.*	2	0
Sarcoma	1 ml.	2	1
First passage of sarcoma in new-born rats. Removed on sixteenth day and irradiated with 5,000 r.	1 ml.	3	2

* Undiluted whole blood from the cyst.

In the case of the cyst, the presence of virus in the cyst walls, blood and tumour was shown after transfer of these tissues to chickens (see Table 1). In the case of the sarcoma, the blood remained ineffective, thus excluding the possibility of a mechanical transmission of the virus into the sarcoma. The virus was found to be present in the first passage of sarcoma made on newborn rats and taken on the sixteenth day after transplantation. Before transfer to chickens, the tumour tissue was killed by irradiation with 5,000 r. When tumours of the first passage were tested in chickens after 36 days, metastasizing sarcomas were also obtained (results not included in Table 1). In addition, Kryukova[9] found that after infection of rat *M*-1 sarcoma *in vivo* with Rous sarcoma virus, it was impossible to detect the virus after 6 hr. The *M*-1 sarcoma cells incubated with this virus when transferred into rats likewise induced tumours which do not, however, contain the virus. These findings emphasize that in our experiments an intimate association of virus with mammalian tumour cells took place which was promoted by infection of ontogenetically immature recipients.

All tumours obtained in chickens macroscopically and microscopically resembled the Rous sarcoma and contained a large amount of viscous intercellular ground-substance. Their growth was very similar to the growth of Rous sarcoma in non-tolerant[7] and tolerant[8] turkeys. At the site of implantation—in the pectoral region—distinct tumours were observed, but they never reached a large size, because the animals succumbed to a metastatic effusion localized largely in the lungs. The tumours were transferable to chickens both by means of tissue suspension and by extracts obtained through centrifugation at 13,000*g* for 15 min. However, after re-transplantation to newborn rats they did not grow.

All the findings provide evidence that the avian virus may participate directly in the formation of mammalian sarcomas and may be present there in an infectious form.

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RADIOBIOLOGY

Effect of X-Rays on the Development of the Infective Larvæ of *Oesophagostomum radiatum* (Rud. 1803) (Strongylidae : Nematoda)

Following a report by Jarrett *et al.*[1] on the stimulation of resistance in sheep to infestation with *Haemonchus contortus* by dosing with X-irradiated infective larvæ, investigations are being undertaken at this laboratory on the value of this technique in producing resistance in calves to gastro-intestinal helminths.

In a trial with *Oesophagostomum radiatum* in which calves five months old and reared worm-free were used, 4 animals were each given 7,000 normal infective larvæ and 10 others a similar number of infective larvæ exposed to 20,000 r. of X-radiation. The radiation was given in a single dose at 1,000 r. per min. on a 220-kV. 'Maximar' at 220 kV., no filter, H.V.L. = 0·4 mm. copper. Calves from the group given irradiated larvæ were killed and examined for worms 3, 4, 5, 6 and 8 weeks after infestation, and a calf from the control group 6 weeks after infestation.

Table 1. Worms present in Calves given X-Irradiated (20,000 r.) and Untreated Larvæ of *Oesophagostomum radiatum*

Calf No.	Time after infestation (weeks)	Worms present			
		5th stage		4th stage	3rd stage
		♀	♂		
	X-irradiated group				
365	3	160		2,140	0
366	4	920	40	45	0
372	5	465	5	105	0
367	6	268	4	9	0
374	8	1,440	75	0	0
	Control group				
362	6	1,825	1,880	0	0

The results of these examinations are set out in Table 1. Although only one calf in the control group was examined, the proportion of male to female worms in this animal was in conformity with that found in calves used in earlier studies on infestations with untreated larvæ. The presence of fourth-stage larvæ in the calves killed at 4, 5 and 6 weeks is not considered to be a result of radiation, for numbers of this stage have been seen in calves given single doses of untreated larvæ and killed at comparable times. The main effect of this level of radiation therefore was to prevent the establishment of an adult male population in normal numbers.

The fæcal egg counts of the survivors of the control group followed the trend usually seen in experimental infestations, whereas the egg counts of the survivors of the group receiving treated larvæ remained at a low level or were negative. Female worms from the calves of the latter group killed at 5, 6 and 8 weeks contained eggs which appeared to be infertile, and afterwards it was found that most of the eggs in the fæces of the surviving calves of this group could be detected only by sedimentation, as they did not float in the salt solution used in the standard egg counting technique of Roberts and O'Sullivan[2]. No division of the cell had occurred, and the eggs did not develop in fæcal cultures.

In addition to the selective action on the larvæ destined to be males it is possible then that the level of X-radiation used also had a sterilizing effect on one or perhaps both sexes, as recorded by Schwartz[3] for larvæ of *Trichinella spiralis* exposed to X-rays.

Further details of this experiment will be published elsewhere.

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Stimulating Effects of Nuclear Radiations on Development and Productivity of Rice Plants

In a report[1] which came to our notice in 1956, Kuzin showed the presence of the stimulating effects of ionizing radiations on the growth and development of several agricultural crops save rice. It was therefore felt desirable to study a possibility of obtaining an increase in development and productivity of rice crop through treatment with nuclear radiations.

Dry grains of rice varieties *C*33-18 and *D*17-88, the moisture contents of which had been stabilized in a room maintained at about 70 per cent relative humidity for 9 days, were exposed to 500 r., 1,000 r., and 1,500 r. of γ-rays from a 1,000-c. 'Theratron Junior' cobalt-60 teletherapy unit. Germination tests were conducted on moist filter papers in Petri dishes. The sprouts were transferred to nursery pots on the fifth day. When 30 days old, the seedlings were transplanted in unfertilized plots, each measuring 5 ft. by 33 ft. Plant heights and panicle lengths were taken just before harvest, followed by thorough gleaning to make up for the loss due to lodging. The results are summarized in Table 1.

Application of both tagged and untagged mono-ammonium dihydrogen orthophosphate, each in concentrations of 20 lb. phosphate/A., 40 lb. phosphates/A., and 60 lb. phosphate/A., to 6 plots of 39-day old transplants of rice variety *C*28-16 at the ground-level, was made on the ninth day after transplanting; the specific and total activities used being 175 μc./gm. total phosphate and 4554·82 μc., respectively. The results for plant height and grain yield are presented in Table 2.

From Table 1 it appears that germination percentages of both control and irradiated seeds are the same in *D*17-88 and almost equal in *C*33-18. The yield response of *C*33-18 is positive and progressive at all three dosages, whereas that of *D*17-88 at 500 r. and 1,000 r. is positive, but regressive at the latter, and negative at 1,500 r. of γ-radiation. Over the range tested, dosages of 1,500 r. and 500 r. of γ-rays seem to be the best for the highest crop return of *C*33-18 and *D*17-88, respectively. Generally, no clear-cut differences in panicle length and plant height among the control and irradiated *D*17-88 are obtained. Apparently, there is some conspicuous difference only in plant height between control and irradiated *C*33-18, especially at the 1,000-r. level.

Table 1. Effect of γ-Rays on Seed Germination, Plant Height, Panicle Length and Grain Yield of Rice Varieties *C*33-18 and *D*17-88 tested in 1959

Variety	Treatment	Germination (per cent)	Average plant height (cm.)	Average panicle length (cm.)	Grain yield (kgm./A.)	Ratio to control (per cent)
*C*33-18	0 r.	95	129·5	22·2	1,260	100·0
	500 r.	94	134·6	22·5	1,320	104·8
	1,000 r.	96	141·8	22·9	1,371	108·8
	1,500 r.	94	131·8	22·6	1,464	116·2
*D*17-88	0 r.	100	125·3	29·1	1,160	100·0
	500 r.	100	125·4	28·9	1,285	110·8
	1,000 r.	100	129·0	29·8	1,219	105·1
	1,500 r.	100*	124·3	26·7	1,035	89·2

* Shortest plumule and longest radicle.

It is worth recording an observation that the number of plants lodged progressively increased from a very few at the 1,500 r. level to 100 per cent in the control in both varieties just before the maturity of grains. Irradiation treatment therefore seems to promote silicification of cell walls, that is to say, structural strength, through its favourable influence on utilization by rice plants of potassium and/or silicon from the soil. This point is being further studied.

Table 2 indicates that *C*28-16 yields considerably better and higher at all three levels of radioactive phosphate additions than at the corresponding levels of non-active phosphate applications. An increase in the yield of grain dropped by 16–17 per cent due probably to a relatively large total activity at the level of 60 lb. phosphorus-32 phosphate/A. A noticeable gain in stature over plants treated with phosphorus-31 is found among plants subjected to 748·36 μc. and 1517·92 μc. of phosphorus-32. Although phosphorus-32 has a short half-life of 14·30 days, and its activity in plant body and the soil is no longer readily detectable about 3–4 months after fertilization, it cannot as yet be recommended for practical applications in view of the serious problems of radiation protection and health hazards.

Table 2. Effect of Phosphorus-31 and Phosphorus-32 on Plant Height and Grain Yield of Rice Variety *C*28-16 tested in 1959

Treatment*	Total activity of ^{32}P† (μc.)	Average plant height (cm.)	Grain yield in lb. per 23 clusters	Increase (per cent)
0	—	64	0·40	—
20 lb. $^{31}P_2O_5$/A.	—	102	0·87	—
40 lb.	—	113	1·57	—
60 lb.	—	128	2·45	—
20 lb. $^{32}P_2O_5$/A.	748·36	114	1·27	46
40 lb.	1,517·92	123	2·27	45
60 lb.	2,288·54	127	3·16	29

* Applied 9 days after transplanting.
† Calibrated and supplied by Radiochemical Centre, Amersham, Bucks.

These results are clearly indicative of the stimulative effects of comparatively low levels of both β- and γ-rays on the growth and productivity of rice plants. The varietal difference in reaction to an equal dose of the same kind of nuclear radiation is observed. Future work is planned to test the stability or otherwise of these effects in the following season, and the overall interaction between γ-ray treatment of dry seeds over a wider range of doses, and the non-active or radioactive phosphate additions to be made later at various stages of plant growth from irradiated seeds of rice.

Thanks are due to U Hla Nyunt, director of the Union of Burma Atomic Energy Center; U Khin, director of the Department of Agriculture, and U San Khin, chief research officer, Agricultural Research Institute, for their support in the course of this work.

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A Comparison of Iodine-124 and Iodine-131 Thyroid Dosimetry

THE purpose of this communication is to show that the approximate estimate given by Doniach and Francois[1] of the ratio R of the quantities (in mc.) of iodine-124 to iodine-131 required to give the same average dose (in rads) to rat thyroids has been modified by more exact calculations; further, to demonstrate the effect of some biological variables on this ratio; and to discuss the implications in treatment of human thyroid disease with radioactive iodine. There are two such implications. First, the average doses within the human thyroid can be calculated for iodine-124, within certain limits of error, because the maximum and minimum do not differ by more than about 3 : 1. For iodine-131, however, with β-particles of much shorter range, the extreme non-uniformity of dosage, varying from 10 : 1 to 100 : 1 in thyroid tumours[2,3], would appear to render such estimates of 'average dose' of limited use. Iodine-124 would therefore be a therapeutic tool of greater precision than iodine-131, both for the control of thyrotoxicosis and for thyroid carcinoma. The accurate prediction of the amount of isotope required to be administered, which is possible, but is not always thought necessary for iodine-131, would be more worth while for iodine-124.

The second implication arises from the possibility that a smaller 'average physical dose' of relatively uniformly distributed radiation is required to produce the same biological effect in the rat thyroid than a given 'average dose' from iodine-131. Doniach had previously found a gain factor F of about 5 : 1 in rats for X-rays relative to iodine-131, after allowance for the different duration of irradiation, both in the inhibition of thyroid hyperplasia and in the induction of thyroid tumours[4,5]. Such gain factors should be enhanced in the human thyroid. The ratio of the millicurie quantity of iodine-124 to that of iodine-131 required to produce a given biological effect in a thyroid gland is not simply the ratio R of the 'average' physical doses, but is R/F. The factor F will be greater than unity if uniform radiation is more effective than the same 'average dose' of non-uniformly distributed radiation. A more relevant value of F may now be derived from the experiment of Doniach and Francois[1], since this experiment compares the effect of the two internally administered isotopes in rats.

Values of R (between 1·0 and 1·7) have been discussed[6,7] for human thyroids but values of F have yet to be determined.

To Doniach and Francois's experimental finding[1] of $R/F = 1{\cdot}8$ ($\pm 0{\cdot}2$ standard deviation) in rats, we wish to add that the theoretical ratio is $R = 2{\cdot}29$, if the following assumptions are made. Variables are discussed below. The shape of the thyroid gland was assumed to be a pair of cylinders each 5 mm. long and 2 mm. in diameter, of total weight 31·4 mgm., with centres 5 mm. apart, except where otherwise stated. The biological half-life was taken as 100 hr. The cylindrical shape gives rise to computational problems, the solution of which will be described elsewhere[7]. Allowance was made for radiation not absorbed within the gland. The k-factor of iodine-124 was taken to be 7·0 (refs. 8–10). The proportion of iodine-126 present as a contaminant of the iodine-124 was assumed to be 0·22 : 1 (in mc.), so as to be consistent with the material used by Doniach and Francois. The nuclides iodine-123 and iodine-125, in the amounts which were present, would not affect these results. The dose at the centre of one spherical lobe due to the other lobe was estimated to be less than 1 per cent and so this effect was neglected.

The biological half-life affects these results as shown in Table 1.

Table 1

Biological half-life	R (Iodine-124 mc./Iodine-131 mc. to give equal average physical doses in rads—2 cylinders)
2·9 days	2·22
4·17 days	2·29
5 days	2·30
10 days	2·40
Infinite	2·44

The fact that the maximal uptake is not achieved instantaneously would only increase these ratios by about 2 per cent.

Variation in the shape of the organ will affect the ratio as shown in Table 2.

Table 2

Shape	R (biological $t_{1/2}$ 5 days)
Two cylinders as above (31 mgm.)	2·30
Two spheres (total 30 mgm.)	2·12
Single sphere (total 30 mgm.)	1·88

Variations in weight of the rat thyroid will affect the results. The values given in Table 3 have been calculated for a pair of spheres[11], since this model shows the magnitude of the variation with sufficient accuracy.

Table 3

Weight of pair of spheres	R (biological $t_{1/2}$ 5 days)
20 mgm.	2·21
25 mgm.	2·19
30 mgm.	2·12

It will be seen that the extent of these variations is not unduly large compared with other errors in such experiments.

It should be mentioned that the proportion of iodine-126 present affects the calculated ratio R. This factor is not, of course, a biological variable, and the proportion present in any specimen of iodine-124 can be determined. In Doniach and Francois's experiment, the accuracy of this determination was ±5 per cent, and the change in R resulting from a change in proportion of iodine-126 from 0·21 : 1 to 0·23 : 1

would be from $R = 2{\cdot}32$ to $R = 2{\cdot}26$. (Subsequent batches of iodine-124, produced by bombardment at 30 MeV. instead of 18 MeV., have contained much smaller proportions of iodine-126.)

The general conclusions are not affected by such variations as are likely to occur. In particular, the experimental ratio for R/F of $1{\cdot}8 \pm 0{\cdot}2$ (excluding the k-factor uncertainty) found by Doniach and Francois is lower than the calculated value of $R = 2{\cdot}29$, but the difference is only about twice the standard deviation of the experimental results. This shows that the gain factor F must be between 1 and 1·4. Therefore if the better uniformity of dose distribution affects this result *per se*, then the effect is small compared with the known non-uniformity of concentration of iodine-131 in rat thyroids[12], which was 5 : 1 for individual follicles. However, these follicles are of the order of 0·05 mm. diameter so that the dose in rads is likely to vary by less than 2 : 1 even for iodine-131. Therefore the experimental value of F is not inconsistent with the dosage non-uniformity factor in rat thyroids. The results of the comparison of X-rays and iodine-131 referred to above[5] depend upon other factors, that is, dose-rate, duration of exposure, and lower iodine uptake and dosage at the periphery of the glands. They are likely to be less relevant than a comparison of two isotopes.

In human thyroid tumours, however, there are areas up to 5 mm. diameter with little or no uptake of iodine[6], so that here F may be much greater.

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BIOLOGY

Are Macromolecules Required for Growth of Single Isolated Mammalian Cells ?

A few strains of mammalian cells have been grown from large inocula in a protein-free medium[1]; but the multiplication of single isolated cells requires that the medium be supplemented with protein, usually supplied as whole or dialysed serum[2]. Recently, it has been shown that for HeLa cells the serum can be replaced by two purified proteins, fetuin and albumin[3]. However, the role of these proteins is obscure. The following experiments demonstrate that for one cell strain, at least, the role of serum is not to supply protein as such, but probably to act as a source of essential growth factors of low molecular weight.

Horse serum was dialysed aseptically on a shaker at 5° C. for 24 hr. against ½ vol. of Parker's chemically defined medium, *CMRL*-1066 (phenol red omitted)[4]. In some experiments several such 24-hr. dialyses were carried out on the same sample of serum. *L* cells (strain 929 *E*) were immediately plated in the dialysates, using a procedure described previously[5]. Fresh dialysate was used for feeding the cultures, after one and two weeks of incubation.

While in *CMRL*-1066 alone, the cells failed to undergo even a single division; after incubation for five days in the dialysate most cells had grown into microcolonies. Cell multiplication continued until these colonies contained several thousand cells each. The plating efficiency was about the same as with 20 per cent serum (Table 1). Growth-rates were about the same in both media.

Table 1. Plating Efficiency of *L* Strain Cells in Media with and without Macromolecular Components

	Plating efficiency*		
	Exp. 1	Exp. 2	Exp. 3†
CMRL-1066 + 20 per cent whole horse serum	69	82	73
CMRL-1066 + 20 per cent dialysed horse serum	70	80	69
CMRL-1066	0	0	0
CMRL-1066 dialysed‡ against whole horse serum	65	70	73

* Percentage of 100 cells added to each Petri dish which formed macroscopic colonies.
† In experiment 3 the serum was subjected to two previous 24-hr. dialyses.
‡ Whole horse serum was dialysed against one-half volume of *CMRL*-1066 for 24 hr. at 5° C.

In the presence of serum the cells exhibited many cytoplasmic granules, were roughly polygonal in shape, and closely adherent. In striking contrast was their appearance in the dialysate. Here the cells contained relatively few granules, were extremely fusiform, and were seldom in contact with one another. This tendency for cells in the dialysate to separate from one another was also noted in attempts to adapt *L* cells to grow continuously from large inocula in *CMRL*-1066 alone. Possibly this behaviour may be characteristic of growth in a protein-free medium.

When the standard 24-hr. dialysis was repeated three times on the same sample of serum, the final dialysate gave as high a plating efficiency as the first (Table 1, Exp. 3). This result would be expected from the equivalence of whole and dialysed serum in supporting cell growth. However, the amount of active material at any time was small, since even a twofold dilution causes a marked (fivefold) drop in plating efficiency. Dialysates did not give the biuret reaction, indicating that the concentration of peptides, if present, was low.

These experiments demonstrate that serum as such is not required for the multiplication of single *L* cells. It is possible that dialysis against serum may adjust the concentrations of the constituents already present in *CMRL*-1066, but it is more likely that serum is supplying one or more essential dialysable growth factors not present in the synthetic medium.

Some batches of horse serum have failed to yield active dialysates. A possible reason for this is advanced in a paper by Eagle[6] which appeared as this communication was being written. His experiments show that cultures inoculated with 10^5 *L* cells per ml. failed to grow in a chemically defined medium separated by a 'Cellophane' membrane from dialysed serum unless

proteolytic enzymes were added to the serum compartment. This suggests that growth factor(s) was released by breakdown of the serum proteins. Since proteolysis of serum varies with temperature and time of storage[6], neither of which was controlled in my experiments, such variation could explain my failure to obtain active dialysates in some instances. This hypothesis is being tested. If, indeed, proteolysis is responsible for the release of growth factor(s), then trace amounts released without added enzymes might allow growth of small numbers of isolated cells, but be inadequate to support the dense populations employed by Eagle.

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Non-specific Tolerance of Graft and the Dissociation of Two Types of Immunity

PREVIOUS work with induced immunological tolerance of skin grafts presented evidence of a strict specificity of acquired tolerance. The 'non-specific' tolerance chould be induced in ducks by injecting them with a mixture of bone marrow and spleen cells from 60 pooled donors[1]. This finding, as well as other observations of 'non-specific' tolerance[2] can be explained by the antigenic overlapping between individuals genetically related to a certain extent. However, it was shown that, when heterografts were tolerated, tolerance of heterografts from other individuals of the same species could be also obtained. In one instance, tolerance of a turkey homograft[3] was found in a turkey which tolerated two chicken heterografts.

In chickens that had been in embryonal parabiosis with turkeys, the tolerance of skin heterografts in postfœtal life is of a much lower degree than that observed in turkeys. We have therefore sought to find out whether these animals would be able to tolerate chicken homografts even though they did not tolerate, or only partially tolerated, the turkey heterografts. In seven chicken parabionts, skin heterografts transferred at 14 days of age never survived longer than 20 days after transplantation. However, in five chickens, homografts continued to be tolerated. On four of them feathers grew, and the first signs of their destruction (feathers falling off, minor necrosis) were observed 35–60 days after transplantation. The destruction proceeded slowly and the final destruction of homografts was very difficult to determine (Fig. 1).

It does not seem probable that the heterologous antigenic complex would involve the antigens which are necessary for the induction of homologous tolerance. Hašková (unpublished work) did not find

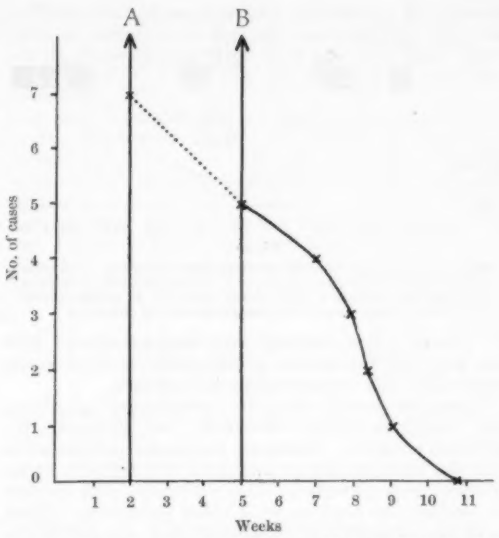

Fig. 1. Occurrence of the first signs of homograft destruction in parabiotic chickens with partial heterologous tolerance. *A*, Transplantation of skin at two weeks after hatching; *B*, the limit of maximal survival of homografts on control chickens and of heterografts on parabiotic chickens

that rat heterografts would induce immunity in *CBA* mice to skin homografts from *C* 57 and *A* strains of mice. Donors selected at random for homotransplantations in tolerant animals, and the persistence of homografts after the heterografts were sloughed, are inconsistent with the fact that individual specific antigens possessed in common by the two species might be involved in inducing this type of tolerance. It can be assumed that all heterologous cells were eliminated from the organism by the immune reaction and together with them all the sources of heterologous antigenic stimulus. The destruction of heterografts likewise shows that the animals are capable of immunological response. It seems, therefore, more probable that this non-specific immunological tolerance of homografts is due to a somewhat more complex mechanism. The heterologous antigenic complex induces in the embryo simultaneously and in even a higher degree the tolerance to homologous antigens which are not present.

In addition, the dissociation of two types of immunity has been found in tolerant animals, namely, transplantation immunity to skin grafts and antibody formation against erythrocytes, which is analogous to that found by Štark, O., Frenzl, B., and Křen, O. (personal communication) in homologous relationships in chickens. In turkey parabionts with a chick, the chicken heterografts are frequently tolerated for several months. In Fig. 2 one such case is included; the chicken graft tolerated by the turkey had been transferred to it 14 days after birth. The interspecies blood mosaicism disappeared from the turkey's circulation within two weeks after hatching. Between the eighth and twelfth week after hatching, immunization of animals with chicken blood was produced and around the graft repeated local applications were made of a large amount of turkey antiserum against chicken blood. The skin graft from the partner survived in spite of the fact that the recipient produced agglutinins against chicken erythrocytes of

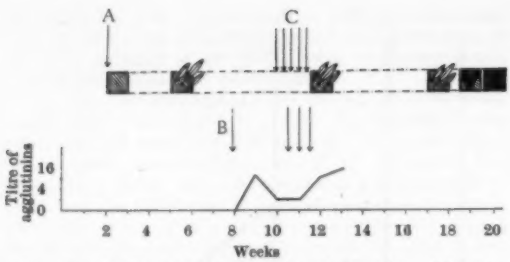

Fig. 2. Behaviour of a heterograft and formation of hetero-agglutinins in a parabiotic turkey. *A*, Transplantation of chicken skin from the partner to a parabiotic turkey; *B*, immunization with chicken blood; *C*, passive transfer of antiserum

the partner after starting with immunization. The first signs of destruction of the graft occurred seven weeks after the immunization had ended.

It may be possible that the formation of agglutinin and transplantation immunity are induced by different antigens; however, it seems more probable that the identical antigen releases two different types of immune mechanism. This difference may be due to binding the antigen with other substances which have no antigenic determinants, but do affect the further fate of the antigenic stimulus. Moreover, it appears that in heterologous relationships, the circulating antibodies do not disturb the tolerance of skin grafts, and the repeated local application of large amounts of heterologous serum (28 ml. within 9 days, the titre being 1 : 32) obtained by blood immunization fails to result in graft destruction. As in the case of a homograft[4], the serum antibodies do not induce the destruction of a tolerated graft.

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Retarded Development of *Ostertagia* Species in Sheep

Sommerville[1,2] has indicated that the immature stages of *Ostertagia* species in sheep may remain dormant or slowly developing over a considerable period within the abomasal pits and glands. The factors controlling this delayed development have not yet been elucidated.

Experiments have been carried out to determine whether the size of a single infesting dose will influence the percentage of larvæ remaining in a partially developed state. Young sheep that had been raised 'worm-free' (excepting for *Strongyloides* species) were used. They received by a flank injection into the rumen either 1,000 or 100,000 infective larvæ. Autopsy was carried out 14 days later and a peptic digestion technique was used to ensure complete recovery of larvæ within the abomasal mucosa. The length was measured of at least 200 or 400 developing worms at the low and high dose-level respectively.

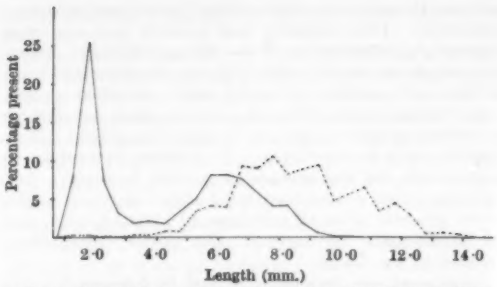

Fig. 1. Composite frequency polygon of size-distributions within the two groups of sheep. All sheep killed at 14 days. – – –, larval dose 1,000; ———, larval dose, 100,000

At the higher dose-level a clearly bimodal size distribution appeared in each of the three lambs in this group. Of the developing larval worms 35–75 per cent were only 1–2 mm. in length; relatively few were 3–5 mm. and the remainder were grouped within and around the 5–8 mm. range, as seen in the accompanying composite frequency polygon prepared by combining the data from within each group of sheep (Fig. 1). Accurate mean, modal or median points cannot be obtained from these measurements because data from male and female worms were combined and the modal point for each sex is different.

At the lower dosage-level less than 2 per cent of developing worms were retarded in their growth, the great majority giving a more or less normal distribution around the 6–11 mm. size range.

Two features became apparent from these results, as shown in Table 1 and Fig. 1. At the higher dose-level a significantly greater ($P < 0{\cdot}01$) percentage of larvæ had not attained 4 mm. in length in 14 days than at the low dosage-level. It appeared that the 'retarded' larvæ had failed to recommence growth following the third moult within the abomasal mucosa. This group consisted entirely of very small fourth-stage larvæ. Secondly, those larvæ at the higher dose-level that had developed beyond the 1–2 mm. size had still failed to attain the length achieved by the worms resulting from the low-level dose.

In the same experiment two additional sheep were given 100,000 larvæ and similarly autopsied at 56 days. It can be seen from Table 1 that although the total number of worms recovered at 56 days was similar to that recovered at 14 days, there was a great reduction in the proportion less than 4 mm. in length. It appears that retardation is not necessarily permanent and that growth and development may commence after a variable period of time. The limited data available also appear to support the suggestion that once growth has recommenced in the

Table 1

Sheep No.	Dose of larvæ	Days to autopsy	Dose recovered at autopsy (per cent)	Recovered larvæ less than 4 mm. (per cent)
*C*921	1,000	14	94·6	1·9
*C*939	1,000	14	92·6	1·1
*C*926	1,000	14	100·0	1·6
*C*930	100,000	14	56·7	35·9
*C*949	100,000	14	27·1	75·8
*C*944	100,000	14	32·4	59·1
*C*928	100,000	56	29·9	21·5
*C*927	100,000	56	27·2	19·6

retarded larvæ they develop rapidly to reach the same length as the others which showed a 'normal' development. It is noteworthy, however, that even at 56 days members of the high-dose populations had not reached the size of the worms in the low-dose populations, and had not, in fact, achieved the normal size quoted for *Ostertagia circumcincta*, the predominant member of the genus in the population. Despite their small size these worms appeared morphologically to be sexually mature and eggs were visible within the uteri of the females. Only relatively low and erratic egg counts (rarely more than 1,000 eggs per gram) were observed in these sheep, despite their heavy worm burden.

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Boron Deficiency in *Grevillea robusta*

Grevillea robusta A. Cunn. is used as a shade tree in many tea plantations in Kenya. It is easily established, and is little subject to pests or diseases. In December 1959 it was reported that *Grevillea* were dying on the whole of one field in a tea plantation in the Nandi Hills district of Kenya. The trees were 2–3 years of age and were dying back from the tip. Of many, only the main branches near the apex of the tree were affected, while one or two lateral branches lower down the tree carried healthy leaves. The more seriously affected trees were almost completely defoliated; but leaves could in many cases be seen sprouting from the main trunk at heights of 4 ft. down to ground-level. In almost all trees showing advanced symptoms there was cracking of the bark and exudation of gum. The symptoms are similar to those described by Vail *et al.*[1] for boron deficiency in wattle. The fact that, prior to the planting of tea and trees of *Grevillea* in the area in question, an attempt to establish wattle had failed, led to an investigation into the boron status of the soil and of the *Grevillea* trees.

Leaf samples were taken from two dead trees, some apparently healthy trees in the same field, and some healthy trees about two miles away from the affected field. Boron analyses on these samples showed one healthy leaf to have the highest boron content (17 p.p.m.) and one from a diseased tree to have the least (13 p.p.m.); the results of further leaf analyses were somewhat inconsistent. The figures for water-soluble boron in the soil, however, supported the idea that boron deficiency was in fact responsible for the effects. Table 1 summarizes the relevant results.

Soil samples taken at a depth of 18–24 in. from a healthy area in the Kericho district some sixty miles to the south of Nandi Hills had a water-soluble boron content of 0·13 p.p.m.

Table 1. Water-Soluble Boron (p.p.m. of Dry Soil)

	0–9 in.	9–18 in.	18–27 in.	27–36 in.
Soil from affected area	0·13	0·07	0·03	0·03
Soil from healthy area	0·20	0·17	0·14	0·06

Treatment by applications of borax to the affected area is being tried and an investigation into the boron contents of soil in other parts of East Africa is proceeding. A fuller report will be published elsewhere.

It may be noted that tea bushes showed no symptoms, which may be due to their lower boron requirement[2], or more probably to the effectual 'scavenging' operations of the extensive tea root system.

My thanks are due to the Board of Management and the Director of the Tea Research Institute of East Africa for permission to publish this communication.

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Effects of *Gibberella fujikuroi* on Fungal Root-infections of *Pinus*

In connexion with several communications in *Nature*[1–4] concerned with the effects of gibberellins on the nodulation of legumes, it would seem opportune to put on record the following experimental observations relating to the influence of *Gibberella fujikuroi* on certain other root associations.

The test plants were seedlings of *Pinus sylvestris* growing in pot-cultures with three different soils, namely, two podsols (*p*H 5) contrasting as regards their mycological contents, and a soil of the agricultural type (*p*H 6·8). *Gibberella* was inoculated into the soils at sowing, and re-inoculated twice at monthly intervals. Three months from sowing, the inoculated cultures started to exhibit a slight depression in development. The figures in Table 1 give the average heights of seedlings five months old.

Table 1

	Podsol *A* (cm.)	Podsol *B* (cm.)	Agricultural soil (cm.)
Plants from control cultures	6·0	5·2	4·9
Plants from inoculated cultures	5·7	4·4	4·4

The decrease in vigour and height of the treated seedlings was accompanied by a noticeable shift in the proportion of the various root-associations affecting the plants. In podsol *A* which, in the control cultures, showed about an equal amount of normal and subnormal mycorrhizas, the presence of *Gibberella* stimulated the formation of the subnormal type of association at the cost of normal mycorrhizas. Decrease in the incidence of true mycorrhizal infection and simultaneous spread of a parasitic association (formed by *Rhizoctonia sylvestris*) was observed for the seedlings in podsol *B* inoculated with *Gibberella*. The 'treated' cultures in the agricultural soil exhibited a marked increase in numbers of a harmful (haustorial) infection and considerable suppression of mycorrhizas.

In older seedlings, about 8 months old, the slight growth differences between controls and inoculated

cultures had disappeared, and examination of the root systems revealed a less pronounced contrast in incidence of mycorrhizal and non-mycorrhizal associations. These findings agree, at least in some respects, with observations by Mes[4] on the effect of gibberellic acid which, when applied to *Vicia villosa* in the very early stages of growth, was recorded to decrease nodulation, but had little or no influence on nodule formation in older plants.

Mixed cultures on a nutrient agar medium (for composition of this medium cf. Levisohn[5]), showed antagonistic effects of *Gibberella* on several mycorrhiza-formers tested, for example, the basidiomycetes *Boletus granulatus*, *B. scaber*, and *Rhizopogon luteolus*, and also on the parasitic mycelium *Rhizoctonia sylvestris*. In this connexion it should be mentioned that gibberellic acid incorporated in the above medium (before sterilization) at a concentration of 100 p.p.m. affected the growth-rate of the mycorrhizal fungi adversely. Colony diameters after 4 weeks growth are given in Table 2. *Rhizoctonia sylvestris* showed a profuse development of sclerotia in the presence of gibberellic acid.

Table 2

	Control medium (cm.)	+ 100 p.p.m. gibberellic acid (cm.)
Boletus granulatus	4	2
B. scaber	15	10
Rhizopogon luteolus	11	6

Detailed observations relating to the effects in soil cultures and nutrient media of *Gibberella* and gibberellic acid will be published elsewhere.

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Sex Ratio of Ammocœtes

In a previous communication[1], it was suggested that variations in the sex ratio of adult brook lampreys (*L. planeri*) might be due to the effect of environmental factors on the course of differentiation in the so-called hermaphrodite gonad of the ammocœte larva. As a result of the examination of a very large number of ammocœte gonads (Table 1), this view is now considered to be untenable.

The material used for this work consisted of ammocœtes of *L. planeri* from the River Yeo (north Somerset); mixed collections of *L. planeri* and *L. fluviatilis* from the middle reaches of the River Usk and from the River Teifi a few miles above the estuary; and specimens of *P. marinus*, the landlocked sea lamprey of the Great Lakes from the Oqueoc River, Michigan.

In *L. planeri*, and probably also in *L. fluviatilis*, differentiation of the gonads is, in a majority of ammocœtes, completed towards the end of the second summer of larval life at lengths of 50–60 mm. In *P. marinus*, on the other hand, recognition of the definitive ovary or testis is not possible in all cases at lengths less than 90 mm. The figures in Table 1 refer, therefore, in the case of *L. planeri* and *L. fluviatilis*, to animals more than 60 mm. in length and for *P. marinus* to those more than 90 mm. Even so there remains a number of animals in which differentiation of the gonad is not complete. In some cases, these transitional gonads are smaller than the definitive ovary and contain both auxocytes and follicles of undifferentiated germ cells; the remainder consists of apparent ovaries in which degenerative changes are occurring involving the disintegration of oocytes and the development of a pronounced connective tissue stroma in the medullary part of the gonad. A close study of sex differentiation in the ammocœte has convinced me that both these transitional gonad types are to be regarded as eventual testes.

Table 1

	Total	♀	♂	Transitional gonads	Ovaries per cent all gonads
L. planeri (River Yeo)	281	162	94	25	57·7
L. fluviatilis and *L. planeri* (River Teifi)	49	25	24	—	51·0
(River Usk)	61	34	13	14	55·7
P. marinus	72	37	30	5	51·4

In none of the groups of animals examined is the number of males greater than the females, even when all those with indeterminate gonads are counted as males. In the large group of brook lamprey ammocœtes there is a small but significant excess of females (57·7 per cent) and this is also true of the material from the River Usk. In *P. marinus* ammocœtes, and in those from the River Teifi, the sex ratio does not differ significantly from equality.

Reports on the sex ratio of adult brook lampreys have almost invariably indicated some excess of males over females, although individual estimates[2–7] have varied widely from near equality to as much as 5 : 1. In a survey of the spawning population of the River Yeo, carried out during 1947–60, I have found an overall sex ratio of 2·05 ♀ : 1 ♂ for a total of 1,492 animals, with annual variations from 1·2 to 3·4. Observations by Applegate[8] on spawning runs of the sea-lamprey in the Oqueoc River have also shown an excess of males, and of the 24,643 animals examined in 1949 only 31·8 per cent were females. Thus the available evidence, both for *L. planeri* and *P. marinus*, points to an approximately equal sex ratio in the larval stage and a definite but variable excess of males in the adult spawning population. It must therefore be concluded that the female suffers a much higher mortality than the male at some stage in the life-cycle between metamorphosis and spawning, while the variations which have been observed within the same population indicate that the factors which are responsible for this increased female mortality must operate with varying intensity at different times and in different localities. Since a similar situation obtains in both parasitic and non-parasitic lampreys it is possible that this increased female mortality may operate during, or shortly after, the critical period when metamorphosis is taking place.

I wish to thank Dr. V. C. Applegate of the U.S. Department of the Interior, Fish and Wildlife

Service for his co-operation in supplying ammocœtes of *P. marinus*.

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Caretta kempi Garman on Welsh Coast

On January 3, a Mexican or Kemp's loggerhead turtle, *Caretta kempi* Garman, was found at high-water mark on Kenfig Dunes in Glamorgan. It was dead, but the body was in a good state of preservation, though the shields of the carapace were mostly missing. The weight was 2,420 gm., the carapace being 244 mm. long and 224 mm. at its maximum width; the length of the plastron was 199 mm. Two other specimens of this species, one slightly larger and one slightly smaller, are in the collection of this Department, both taken alive; one near Freshwater West on the Pembrokeshire coast, in December 1930, the other at Mochras Island at the north end of Cardigan Bay, in November of the same year.

In December 1938, an example of the other species of loggerhead recorded from Britain, *Caretta caretta* (Linn.), was stranded near Tenby; while in February 1946 one weighing 8,180 gm. was found alive on the beach at Oxwich, Glamorgan, and is now in our collections.

Whereas *Caretta caretta* appears to have a fairly wide distribution in tropical and sub-tropical waters on both sides of the Atlantic (though the breeding grounds are on the American side), the natural habitat of the smaller *Caretta kempi* is stated to be restricted to the Gulf of Mexico and adjacent waters, and it is less likely to be carried to the British coasts. About twenty British occurrences of the former, as compared with ten of the latter species, have been recorded[1].

Deraniyagala[2] suggested that the presence of loggerheads in European waters was probably due mainly or entirely to currents, and that they might be of importance as current-indicators; and Parker[3], discussing turtle strandings in 1938 on British coasts, considered it "difficult not to associate their coming with the remarkably warm weather and almost uninterrupted series of south-westerly gales which characterized late November and early December. It seems probable that there has been a strong indrift of warm surface water into the English Channel from the south-west". Russell[4] suggested another possible explanation—the general extension in recent times of warm water farther towards the Arctic, with a resultant northward extension of the boundaries of certain animals. He instanced the capture of a flying fish, *Cypsilurus heterurus*, in Oslo harbour in 1937; and it may be noted also that a *Caretta caretta* was found stranded on the Norwegian coast in December 1951[5]. No loggerhead had ever before been recorded from Scandinavian waters nor from the area east and north of Shetland.

From information supplied by Mr. H. H. Lamb of the Meteorological Office, it would not appear that the warm summer of 1959 over Britain and Europe had any connexion with the present occurrence of *C. kempi* on the Welsh coast. In fact the western two-thirds of the North Atlantic were colder in 1959 than for many years past, only the Bay of Biscay, the North Sea and waters east of about 20° W. being slightly warmer than average. It seems much more likely to be associated with some anomaly of the prevailing water currents. Over the north-eastern Atlantic, especially the waters around the British Isles and farther north, there was a remarkable prevalence of southerly winds in the autumn of 1959. The autumn and early winter, including December, were also notable for the repeated occurrence of a great sweep of west to north-west winds across the whole width of the Atlantic fanning cold water eastwards towards the Bay of Biscay; and this might possibly have produced a flow of water from the Bay of Biscay northwards to the shores of Britain.

Note added in proof. Since the above was written, I have been informed that the British Museum (Natural History) received a specimen of *Caretta caretta* found alive at South Uist on January 8 this year.

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Elminius modestus Darwin in South-West Scotland

The present status of *Elminius modestus* Darwin in the British Isles has recently been summarized by Crisp and Southward[1]. They state that in 1958–59 "it is still relatively rare in Luce Bay and has not yet passed the Mull of Galloway. Similarly, there has been little change in the isolated and sparse colony in Lough [Loch] Ryan at the entrance to the Clyde Sea". In an earlier publication, Crisp[2] states that this Stranraer population, although dwindling in numbers, has persisted since 1950 and consists largely of old animals with corroded shells. According to his classification of abundance, rare is taken as "less than 1/m.^{2}; only a few isolated specimens in 1 hour's search" and occasional as "1–100/m.^{2}; often local and needing to be searched for; rarely close enough to breed".

Over a number of years we have been making an annual survey of the density of settlement of barnacles in the Clyde sea area and our observations on the present status of *Elminius modestus* in south-west Scotland are not entirely in agreement with those quoted above. The following results refer to early August 1959.

Elminius modestus is unevenly distributed in Luce Bay, possibly because of variations in local hydrographical conditions caused by a curious development of shingle spits. Nevertheless, it is readily found in many places, being most common on the western shore; for example, on an artificial structure running across the beach near Sandhead adults at a density of 1/cm.^{2}, young at 8–10/cm.^{2} and spat at 5–10/cm.^{2} were recorded. On parts of the shore at Drummore the species is common with individuals up to 2/cm.^{2}. Furthermore, the species has passed the Mull of Galloway since it was found at Port Logan on the open coast; although not common here, small groups were near enough for breeding if they reached maturity; large adults were not found.

There are at least two well-developed populations in Loch Ryan, with scattered individuals elsewhere on the shores. On the western side near Kirkcolm, *Elminius modestus* is abundant on artificial structures where weeds are infrequent. All size groups from fully grown adults to spat were recorded and in some 'cleared' areas cover is complete. On the town pier at Stranraer, although much less common than at Kirkcolm, all size groups are present and spat was settling in the late autumn. Scattered individuals are present on the railway pier. Larvæ were common in the summer plankton.

A full account of this work will be published elsewhere.

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Multiple Infections of Trematodes in a Snail

In an investigation of the incidence of various larval trematodes in the estuarine snail *Valacumantus* (*Pyrazus*) *australis*, results have been obtained showing that two species of trematodes tend to occur together in individual snails surprisingly often. To my knowledge an observation of this kind has not been recorded before. Cort *et al.*[1] studied the incidence of various larval trematodes in the snail *Stagnicola emarginata angulata*, and from an analysis of more than 7,000 dissections concluded that, in certain instances, there must be an immunity produced by the penetration of one species of trematode which prevents penetration of the same snail by a second species; or that there is an antagonism present which prevents the development of one when the other is present. In other instances of multiple infection they concluded that there was no evidence of any immunity or antagonism. In view of their findings the present results are noteworthy.

Results from an examination of 1,146 snails from Narrabeen Lake, near Sydney, is tabulated below:

	No.	Per cent
Stictodora spp. (Heterophyidae)	269	23·5
Austrobilharzia terrigalensis (Schistosomatidae)	52	4·5
Stictodora/*Austrobilharzia* (multiple infections)	39	3·4
Immature and unidentified infections	16	1·4
Other infections (Echinostomatidae and Philopthalmidae)	169	14·8
Total infected	545	47·6

Several postulates are possible to explain the unexpectedly high incidence of multiple infections of heteropyids and schistosomes. They need not be mutually exclusive: (1) a snail infected with the schistosome is predisposed to infection with the heterophyid; (2) a snail infected with the heterophyid is predisposed to infection with the schistosome; and (3) when the two kinds of eggs occur in the same bird evacuation, which happens to be in close proximity to a suitable snail, infection with the heterophyid and the schistosome occurs simultaneously.

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GENETICS

Apparent Double Crossing-Over in a Short Genetic Interval in *Drosophila melanogaster*

In view of the reported occurrence of double crossing-over within a short genetic interval in *Aspergillus*[1] and of the purported existence of negative interference in bacteriophage[2], it is worth while to record the recovery in *Drosophila melanogaster* of apparent double cross-over types within a short genetic interval where interference is nominally expected to be complete. These double cross-overs were found incidentally to a study of pseudo-allelism at the white (*w*) loci[3]. The relevant genetic interval extends from the *X*-chromosome loci of yellow body colour (*y*) to split bristles (*spl*), mutants of which served as markers for the study of crossing-over between *w* pseudoalleles. The standard map distance for this interval is commonly given as three units, a value at least twice as great as more recent measurements indicate[4]. Throughout this study, parental females were always made heterozygous for the autosomal inversions *Cy* and Ubx^{130}. These inversions serve to increase crossing-over strikingly in the *y*-*spl* interval to values varying from 5 to 11 units[5].

In Table 1 the variety of crosses and detected double cross-overs are listed. Most exceptional individuals were found among the progeny of females heterozygous either for y^3 *su-w*^{a} w^a *spl* (*su-w*^{a} = suppressor of w^a; w^a = whiteapricot eye colour) or *y ac* w^{ch} *spl* (*ac* = acheate bristles; w^{ch} = white-cherry eye colour) since these *w* mutants were used as testers in studying pseudoallelism. No concerted effort was made to recover double cross-overs; rather those tabulated here represent individuals, mostly males, which were easily and effortlessly identified through their distinctive phenotype. At best, therefore, the frequencies noted in Table 1 are minimal. The exceptions are deemed to be double cross-overs because genotypically they represent the expected contrary double cross-over classes: w^a or w^{ch} with none of the marker genes, and the allelic or pseudoallelic mutant with all the markers. Where the former were recovered their genotype was confirmed as follows. The w^a exceptions were tested for suppression by *su-w*^{a}. This is a critical test since this suppressor's action is delimited to the w^a mutant[3]. Since w^{ch} does not show dosage compensation, this property was employed for its identification. Where the latter class of exceptions was detected, the distinguishing phenotypic effects characterizing the

Table 1. Recovered Apparent Double Cross-over within the *y*-*spl* Interval among the Progeny of ♀♀ Heterozygous for Independent *w* Mutants.

All parental ♀♀ were heterozygous for the autosomal inversions *Cy* and Ubx^{130} and were crossed to *y w spl* sn^3 ♂♂

	Genotype parental ♀♀	Genotype double cross-over	Total progeny
(1)	y^3 *su-w*^{a} w^a *spl*/w^{ch}	♂ y^3 *su-w*^{a} w^{ch} *spl*	28,896
(2)	*y ac* w^{ch} *spl*/w^{coi}	♂ *y ac* w^{coi} *spl*	33,684
(3)	y^3 *su-w*^{a} w^a *spl*/w^{a4}	2 ♂♂ y^3 *su-w*^{a} w^{a4} *spl*	90,327
(4)	y^3 *su-w*^{a} w^a *spl*/w^{a3}	♂ y^3 *su-w*^{a} w^{a3} *spl*	63,940
(5)	y^3 *su-w*^{a} w^a *spl*/w^{coi}	♀ y^3 *su-w*^{a} w^{coi} *spl*/*y w spl* sn^3 ♂ w^a	74,840
(6)	y^3 *su-w*^{a} w^a *spl*/w^{coi}	♂ w^a	102,145
(7)	y^3 *su-w*^{a} w^{a4} *spl*/w^a	♂ y^3 *su-w*^{a} w^a *spl*	27,705
(8)	y^3 *su-w*^{a} w^{a4} *spl*/w^{a3}	♂ y^3 *su-w*^{a} w^{a3} *spl*	50,210
(9)	*y ac* w^{ch} *spl*/w^{a3}	♂ w^{ch}	20,065
(10)	*y ac* w^{ch} *spl*/w^{a61}	♂ w^{ch}	54,975
(11)	y^3 *su-w*^{a} w^a *spl*/w^{a61}	♂ w^a	47,110
(12)	*y ac* w^{ch} *spl*/w^{dp3}	♀ *y ac* w^{dp3} *spl*/*y w spl* sn^3	49,670
(13)	y^3 *su-w*^{a} w^a *spl*/w^{a57i}	2 ♂♂ y^3 *su-w*^{a} w^{a57i} *spl*	93,744
(14)	*y ac* w^{coi} *spl*/w^{a3}	♀ w^{coi}/*y w spl* sn^3	195,870
(15)	*y ac* w^{ch} *spl*/sp^3-*w*	♀ *y ac* sp^3-*w spl*/*y w spl* sn^3	92,400
(16)	*y* $w^{a2}E$ *spl*/w^a	♂ $w^{a2}E$	79,430
(17)	y^3 *su-w*^{a} w^a *spl*/$w^{a2}E$	♂ w^a	93,670

w mutant served, in most instances, to verify its genotype. For example, the mutants w^{col}, w^{sal} and sp^{3}-*w* have such distinctive phenotypes and are mutationally so rare in occurrence that their recovery in association with all marker genes is best accounted for in terms of a double cross-over occurring on each side of the *w* loci.

Since the results do not represent a quantitative recovery of double cross-overs, there is little point in indulging in computations of the degree of interference. What these results do demonstrate is that, for a comparative short genetic distance, interference is not necessarily complete and double cross-overs do infrequently occur.

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Thread Protein and β-Globulin Polymorphism in the Serum Proteins of Pigs

THREAD proteins, reported to be polymorphic in the sera of cattle[1], have now been demonstrated in serum samples from pigs, dogs, and horses. Thread protein polymorphism has been found in the pig also, but not in the relatively few samples from dogs and horses examined up to the present.

Pig thread proteins are readily seen in one-dimensional starch-gel electropherograms using the same *p*H 7·6 phosphate buffer and procedure described for cattle sera[2]. The zones are very narrow, indeed thread-like, in suitable gels. However, the zones may be faint, or not appear at all if the nature of the gel deviates only a little from the optimum. No difficulty has been experienced in detecting thread proteins in gels prepared from several batches of Hopkin and Williams potato starch after suitable acid hydrolysis[3]; but it has not been possible to detect thread proteins, particularly in cattle sera, using a commercially available starch hydrolysed ready for starch-gel electrophoresis.

Three thread-protein phenotypes have been found in sera from Large White and Essex Saddleback pigs, depending on the presence of one or both of a pair of thread proteins. The three phenotypes are shown in Fig. 1, after two-dimensional electrophoresis, first in agar then in starch gel. Sera of the three phenotypes were resolved initially in 1 per cent agar using *p*H 8·4 borate buffer (4·65 gm. boric acid in water adjusted with sodium hydroxide and diluted to 1 l.). Afterwards 0·5 cm. wide length-wise strips of agar containing the resolved albumin zone were cut from each of the three agar gels and inserted side-by-side across a previously prepared starch gel of suitable width. After electrophoresis the starch gel was sliced with fine wire and stained in nigrosine[3]. Fig. 1 shows that the agar albumin zones also contained some α-globulins, the thread proteins and a pre-albumin zone.

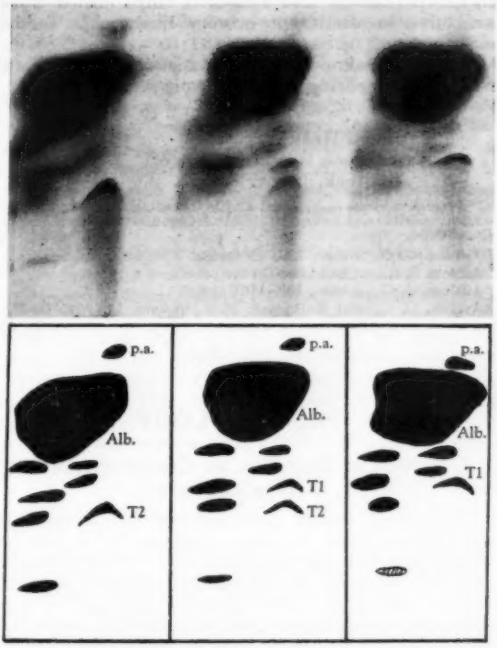

Fig. 1. The three thread-protein phenotypes in pig sera. The illustration shows the agar electrophoresis resolved albumin zones after further resolution in starch gel. The thread-protein phenotypes of the three serum samples are, from left to right, *T*2, *T*1/*T*2 and *T*1. In order to emphasize the thread proteins the starch gel was somewhat overloaded, resulting in poor resolution of the α-globulin zones. *p.a.*, Pre-albumin; *Alb.*, albumin; *T*1 and *T*2, thread proteins. Remaining zones are α-globulins. The β- and γ-globulins were not presented to the starch gel

The nature of the thread proteins is not clear. In all animals in which they have been found they occupy the same relative position after two-dimensional electrophoresis, that is, at the leading edge of the albumin zone in agar electrophoresis, and well separated from the α-globulins in the second starch-gel electrophoresis. In two-dimensional electrophoresis the thread protein zones usually appear crescent-shaped, a phenomenon not shown by the remaining zones.

Preliminary mating data suggest that the three pig thread-protein phenotypes comprise the two homozygotes and heterozygote of a two-allele system. By analogy with the thread-protein system in cattle it is suggested that the two alleles be named pig T^A and T^B, so that the faster migrating zone *T*1 is seen in the presence of T^A and the slower zone *T*2 is seen in the presence of T^B. The three genotypes T^A/T^A, T^A/T^B and T^B/T^B would then be represented by phenotypes *T*1, *T*1/*T*2 and *T*2 respectively.

In common with all other mammals so far examined pigs also exhibit β-globulin polymorphism. Three β-globulin phenotypes have been seen in the samples examined, comprising the two homozygotes and heterozygote of a pair of β-globulin alleles, pig β^A and β^B. Each allele gives rise to three zones in starch gel in phosphate buffer, a rather faintly staining zone followed by a moderately staining zone and then by a more intensely staining zone. This is very similar to the situation found in sheep[3], goats[4] and mice (Ashton and Braden, unpublished work). In view of the increasing numbers of β-globulin alleles being found in other mammals, to date five in humans[5], ten

in sheep (Ashton and Ferguson, unpublished work) and five in cattle[6], it is very likely that further examples will be found in pigs.

I wish to acknowledge the assistance of Dr. K. C. Sellars in procuring samples of pig sera.

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MICROBIOLOGY

L Forms of Bacteria as Contaminants in Tissue Culture

The occurrence of pleuropneumonia-like organism as contaminants in tissue culture is a common experience in many laboratories[1–5]. Such infections may be inapparent or may cause progressive degeneration of the cells. The origin of this type of contamination has never been satisfactorily explained, and although the serum used in tissue culture media is often suspected we have never succeeded in isolating pleuropneumonia-like organisms from many samples of fresh human, bovine, horse and rabbit sera. Our results suggest that at least some of the pleuropneumonia-like organisms found in tissue culture are *L* forms of contaminating bacteria. This belief is based on the following observations.

Twenty cell lines carried in our laboratory yielded minute colonies when the medium in which they had grown was plated on soft meat digest agar containing 20 per cent heated horse serum. A complete minute colony derived from a HeLa culture was inoculated into 5 ml. of meat digest broth containing 20 per cent horse serum, 10 per cent yeast extract, and 100 units of penicillin per ml. This was sub-cultured and when slightly turbid a loopful was stroked out on the solid medium used for the initial isolations. Tiny colonies appeared following incubation for 2 days at 37° C. On the third day most of the small translucent colonies had produced further dense opaque outgrowths. These were found to be composed of a penicillin-sensitive *Corynebacterium*. It was clear that these *Corynebacteria* were derived from the small colonies. Pure cultures of the *Corynebacterium* and its *L* form were maintained in 20 per cent horse serum broth. Penicillin was always added to the *L* form cultures to prevent reversion to the bacterial form.

In order to test the hypothesis that the *Corynebacterium* gave rise to its *L* form in tissue culture, HeLa cultures were cured of *L* form infection by growing them for several sub-cultures in medium containing 200 units of neomycin ('Mycifradin sulphate', Upjohn Co.) per ml. Preliminary experiments had shown that the *L* forms from several cell lines were very sensitive to the tetracycline group of antibiotics and neomycin.

Terramycin, chloramphenicol and aureomycin were found to be toxic for the cells at effective concentrations. However, Hearn *et al.*[4] have used aureomycin at toxic levels to free tissue cultures from pleuropneumonia-like organism contaminants. This hazardous procedure was avoidable since neomycin was effective and had no observable toxic effect on HeLa cells at a concentration of 200 units/ml. At each sub-culture during neomycin treatment some cultures were grown without antibiotics for several sub-cultures with frequent changes of medium. When repeated tests for *L* forms in the medium were negative, the cells were deemed to have been cured. Up to 14 days treatment was necessary in some cases. It is of interest that the cells had to be maintained in medium containing 200 units of neomycin per ml. for several sub-cultures before *L* forms became undetectable, even although the organisms were susceptible to <10 units per ml. of the antibiotic in agar medium. The necessity for this high concentration in tissue culture may be due to poor penetration of the antibiotic into the cell or to some other factor related to the intracellular situation of the *L* form.

Cultures freed from *L* forms were then returned to the original medium with 100 units of penicillin and streptomycin per ml. Some cultures were infected with *Corynebacteria* and others with its *L* form. Although the cells infected with the *L* forms did not appear to be different from the uninfected controls when viewed by low-power microscopy, they had apparently become chronically infected and *L* forms could be isolated regularly from them. In the cultures infected with the *Corynebacterium*, this agent was undetectable in the medium of the infected cultures within 2 days, but persisted intracellularly for 9 days. *L* forms were first detected in these cultures at 6 days and regularly thereafter.

These experiments indicate that contamination of tissue cultures containing penicillin with penicillin-susceptible bacteria may cause an inapparent intracellular infection with the *L* form of the bacterium. This could lead to confusion in antigenic analysis of cells, and in metabolic studies the contribution of the commensal *L* form to the host cells' biochemical activity may be significant and should be excluded.

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VIROLOGY

Reactivation of Poxviruses by a Non-Genetic Mechanism

It has recently been shown that poxviruses inactivated by heat are reactivated in cells in which another poxvirus multiplies[1]. The virus yield from cells infected with inactivated virus of one strain and active virus of another strain contains infectious particles of both strains, and also, if the viruses are sufficiently closely related, recombinants[2]. Inactivation by heat, therefore, does not inactivate the deoxyribonucleic acid of the virus particles, since all the known genetic markers are preserved. Since proteins

are in general less stable to heat than deoxyribonucleic acid, the role of the reactivating virus may be to provide some essential native protein which has been destroyed by heating. On this hypothesis, it should be possible to use, as the reactivating agent, virus of which the deoxyribonucleic acid has been specifically inactivated. In this case the genotype of the heat-inactivated virus only should survive.

Such inactivation might be achieved by treatment with the nitrogen mustard, di(2-chloroethyl)methylamine, which, although capable of combining with protein, reacts preferentially with nucleic acid. Loveless and Stock[3] have shown that nitrogen mustard inactivates phage probably by combination with its deoxyribonucleic acid without affecting the protein structures responsible for adsorption to host cells and combination with specific antibody, and they particularly comment on the low efficiency of multiplicity and cross-reactivation shown by phage treated with mustard.

Preliminary experiments showed that rabbitpox virus (RP*u*$^+$), treated with nitrogen mustard, would reactivate heated RP*u*$^+$, and also heated vaccinia 7*N*, and that the product had the pock character of the heated parent. In order to provide a sensitive test for partial survival of the genome of the mustard-treated parent, the mutants RP*u*1 and RP*u*8 were chosen as the heated parent. The *u* mutants of rabbitpox virus, which produce white non-ulcerated pocks on the chorioallantoic membrane, differ from the wild type (RP*u*$^+$), which produces hæmorrhagic ulcerated pocks, at single genetic sites[4,5]. In mixed infections of the chorioallantoic membrane with RP*u*1 or RP*u*8 and RP*u*$^+$, the wild type outgrows both mutants[5], so that if there is survival of that part of the genome which carries the wild locus it should be readily detected.

RP*u*$^+$ was inactivated by exposure to various concentrations of nitrogen mustard in 0·005 *M* phosphate buffer, *p*H 7·2, for 20 min. at 37°. The excess mustard was then destroyed by a tenfold dilution of the suspension into 5 per cent sodium thiosulphate and further incubation for 2 hr. Assay of particles capable of reactivating heated virus was carried out by inoculating suitable dilutions of the material on the chorioallantoic membrane of 11-day old chick embryos together with 5×10^6 particles of *RPu*1 or *RPu*8 heated at 60° for 12 min.: the heated virus was completely non-infectious[1]. Residual active particles of RP*u*$^+$ were also titrated in the absence of heated virus.

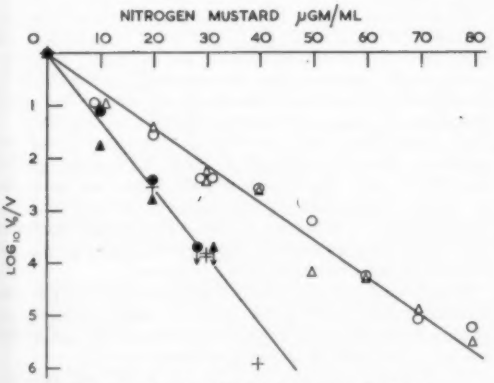

Fig. 1. Inactivation of RP*u*$^+$ by nitrogen mustard. Assayed in the absence of heated virus: +, titre of U$^+$ pocks. Assayed in the presence of heated RP*u*1: ●, titre of U$^+$ pocks; ○, titre of U pocks. Assayed in the presence of heated RP*u*8: ▲, titre of U$^+$ pocks; △, titre of U pocks

The results are shown in Fig. 1. The infectivity of RP*u*$^+$ is seen to be destroyed by nitrogen mustard by a one-hit process, and the surviving particles can be assayed with equal efficiency in the presence or absence of heated virus. The ability of RP*u*$^+$ to reactivate heated RP*u*1 or RP*u*8 is destroyed less readily, though again this appears to be a one-hit process.

It seems, therefore, that in rabbitpox virus all the lethal damages caused by nitrogen mustard prevent expression of the genotype (as judged by one marker), but only about half of them fall in that part of the virus which is necessary for reactivation of heated virus. Thus the evidence is compatible with our hypothesis, namely, that reactivating virus provides some essential non-genetic material which is lacking in heated virus.

We are indebted to Prof. Frank Fenner for the *u* mutants.

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Potato Virus *M* and Paracrinkle

Salaman and Le Pelley[1] found that scions of apparently healthy plants of the potato variety King Edward VII grafted on to supposedly virus-free Arran Victory resulted in the latter variety reacting with paracrinkle symptoms, that is, a pronounced deformity and rugosity of the leaves. Although both Kassanis[2] and Macarthur[3] suggest that King Edward contains only a single virus, Bagnall, Larson and Walker[4] found that a stock of King Edward, which they obtained from Canada and which had been supplied originally by Salaman, contained two viruses. One was a strain of virus *S* and the other resembled virus *M* which they had found in Irish Cobbler and several other American varieties. All normal stocks of Arran Victory were found by Macarthur[3] to contain virus *S*. Therefore, even if there are stocks of King Edward with only a single virus, the paracrinkle symptoms observed in Arran Victory after grafting with King Edward may be due either to the King Edward virus (presumably virus *M*) alone or to the combined action of viruses *S* and *M*, as is suggested by Bagnall, Wetter and Larson[5].

We have also found that King Edward apparently contains two viruses. Thus, for example, sap from King Edward inoculated into fathen (*Chenopodium album*) gave symptoms similar to those from virus *S*-infected Arran Victory, and inoculated on to *Datura metel* gave a systemic yellow spotting followed by an acropetal leaf-drop which resulted in the death of the

plants. No such symptoms were shown by *Datura metel* inoculated with sap from virus *S*-infected Arran Victory.

By grafting King Edward on to the American variety Saco, which is immune to virus *S*, it was possible to obtain plants, the sap of which gave no symptoms when inoculated on to fathen but still produced virus *M* symptoms in *Datura metel*. Scions from these Saco plants produced paracrinkle symptoms in both virus *S*-infected Arran Victory and in plants of a virus *S*-free stock of Arran Victory produced by Kassanis[6] at Rothamsted by the apical meristem culture method. It would appear, therefore, that paracrinkle symptoms in Arran Victory are produced by the virus *M* component alone of the virus complex found in King Edward.

We are indebted to Mr. E. L. Calvert of The Queen's University, Belfast, for the supply of virus *S*-free tubers of Arran Victory.

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AGRICULTURE

Determination of Dieldrin Deposits on Sprayed Mud Surfaces

Chemical investigations into the behaviour of insecticides sprayed on to mud walls require a sampling technique which will remove the insecticide actually present on the surface, and available to the insects, but not that which is sorbed into the bulk of the mud. For laboratory work, Barlow[1] has used 'Sellotape' for this purpose, while Alessandrini[2] has used parchment paper coated with a film of silicone grease. Both these techniques suffer from the disadvantage that the sticky material which is used to pick up the insecticide particles is mounted on a comparatively rigid backing, which prevents its penetrating into the small depressions and irregularities which generally occur on mud surfaces, and which are accessible to insects. Unrealistically low figures are therefore obtained. It was thought that this difficulty might be overcome by the formation of an adhesive film *in situ* on the mud surface, and it has been found that in the laboratory satisfactory films for this purpose can be obtained using solutions containing 1–2·5 per cent w/v of high-viscosity grade (1,500–2,000 cp. for a 1 per cent solution at 25° C.) sodium carboxy-methyl cellulose. The addition of glycerol to the sodium carboxy-methyl cellulose solution is usually necessary; in most cases up to 5 per cent v/v suffices to give a pliable film, but on certain muds up to 25 per cent v/v of this has been necessary to prevent the formation of a hard, brittle film which removes an excessive amount of mud.

The sodium carboxy-methyl cellulose solution is applied to the mud surface mounted on a piece of cotton gauze and should be removed as soon as possible after drying is complete. The film is separated from the gauze by soaking in cold water, and, to ensure complete extraction of the insecticide, the resulting solution is treated with sodium hydroxide and potassium permanganate, and allowed to stand for 30 min. Excess permanganate is removed by careful acidification and treatment with sodium bisulphite. The insecticide is then extracted from the mixture with a suitable solvent, and determined by any convenient method. In the experiments described below, *n*-hexane was used as solvent, and a total chlorine determination using sodium diphenyl reagent was employed. Subsidiary experiments showed no significant loss of dieldrin occurred during the extraction and subsequent treatment.

A series of glass plates and blocks of Uganda mud were sprayed with an aqueous suspension of a dieldrin 50 per cent wettable powder; weighing of the plates before and after spraying showed an average deposit of 114 mgm. dieldrin/sq. ft. Using the above technique, recoveries of 86, 84, 92 and 82 mgm./sq. ft. were obtained from the surfaces of the mud blocks. A similar series of experiments using blocks of Babati (Tanganyika) mud gave recoveries of 74, 83 and 76 mgm. dieldrin/sq. ft., compared with an average deposit of 100 mgm./sq. ft. indicated by weighing of the glass plates. These figures indicate a recovery of between 70 and 80 per cent from both types of mud, and this is adequate to enable sorption to be demonstrated, as shown by the results in Table 1, which were obtained by spraying three series of mud blocks with a dieldrin 50 per cent wettable powder at 65 mgm./sq. ft. and determining the surface deposit at various times after spraying.

Table 1

Days after spraying	Dieldrin recovered (mgm./sq. ft.)		
	Babati	Uganda	Mexico, D.F.
0	54	49	50
1	—	7·0	39
2	—	5·5	—
7	9·7	3·7	24
14	4·0	2·5	15

Results are given for three muds, two of which (Uganda and Babati) have been shown from biological data to be highly active in sorbing dieldrin, and the third (Mexico D.F.) to be less active.

This technique has been tested satisfactorily in the laboratory on a number of different types of mud, although the concentrations of sodium carboxy-methyl cellulose and glycerol required to give the most satisfactory film vary from one to another. Provided that care is taken in removing the film, the amount of mud removed is very small; it has been observed, however, that this is apt to vary with the type of mud used, and this factor should therefore be borne in mind when comparing results of experiments carried out on different muds. The applicability of this technique to muds which give a very loose and friable surface has not been examined.

Thanks are due to "Shell" Research Ltd. for permission to publish this work, and to various colleagues in this laboratory for helpful discussion and assistance with the analytical determinations.

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FORTHCOMING EVENTS

Monday, June 20—Thursday, June 23

LABORATORY APPARATUS AND MATERIALS EXHIBITION (at the Royal Horticultural Society's New Hall, Westminster, London, S.W.1).

Wednesday, June 22

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. R. W. Fairbridge and Dr. Heli Badoux: "Slump Blocks in the Cretaceous of Northern Syria"; Dr. L. J. G. Schermerhorn and Dr. W. I. Stanton: "'Tilloids' in the West Congo Geosyncline".

CHALLENGER SOCIETY (at the Linnean Society, Burlington House, Piccadilly, London, W.1), at 5.45 p.m.—Miss Alison Kay: "The Ecology of the Hawaiian Species of *Cypraea*".

Friday, June 24

VICTOR HORSLEY MEMORIAL FUND (in the Great Hall, British Medical Association House, Tavistock Square, London, W.C.1), at 11 a.m.—Prof. Norman M. Dott: "Brain, Movement and Time" (Eleventh Victor Horsley Memorial Lecture).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SENIOR LECTURER (with a good honours degree and teaching or other suitable experience) TO TEACH ELECTRONICS AND MATHEMATICS to equivalent degree level for sandwich diploma courses—The Principal, Royal Radar Establishment, College of Electronics, Malvern, Worcs (June 22).

ASSISTANT, Grade B, IN MATHEMATICS; and a LECTURER (able to teach to special (honours) degree standard) IN INORGANIC CHEMISTRY—Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (June 24).

SENIOR LECTURER (with high qualifications and wide experience) IN PHYSICS, to teach to honours degree level—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (June 24).

ASSISTANT LECTURER IN NUMERICAL ANALYSIS—The Registrar, The University, Manchester 13 (June 25).

LECTURER (with medical qualifications registrable in this country) IN ANATOMY—The Registrar, The University, Manchester 13 (June 25).

SCIENTIFIC OFFICER (male, with a good honours degree) for genetical, physiological and breeding research in barley—The Secretary, Plant Breeding Institute, Trumpington, Cambridge (June 25).

ASSISTANTS (2) IN ZOOLOGY, one in the field of vertebrate zoology, and the other in comparative physiology—The Secretary of University Court, The University, Glasgow (June 27).

RESEARCH ASSISTANT (with a good degree and preferably a higher degree, in physics, chemistry, engineering or ceramics) AT THE HOULDSWORTH SCHOOL OF APPLIED SCIENCE, to work on the high-temperature properties of certain ceramics of potential interest as engineering materials—The Registrar, The University, Leeds 2 (June 27).

LECTURER IN PSYCHOLOGY or a TEMPORARY LECTURER IN PSYCHOLOGY—The Secretary, The Queen's University, Belfast (June 29).

LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Secretary, The University, Exeter (June 30).

RESEARCH ASSISTANT (with a biological first degree and research experience) IN TISSUE CULTURE, to take part in a programme of research in the behaviour of normal and malignant cells—The Secretary, University College, Gower Street, London, W.C.1 (June 30).

ASSISTANT EXPERIMENTAL OFFICERS, CHEMISTS (with G.C.E. "A" level in chemistry or physics and another science subject or equivalent, if over 22 a pass degree in chemistry or microbiology or H.N.C. chemistry generally expected), at the Water Pollution Research Laboratory, D.S.I.R., Stevenage, Herts, for research on physico-chemical and biological problems in the study and prevention of the pollution of rivers—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. F.384/0A (July 1).

RESEARCH STUDENT (with the degree of Bachelor or Master of a British university other than Manchester) IN SCIENCE—The Registrar, The University, Manchester 13 (July 1).

LECTURER, and a DEMONSTRATOR (honours graduates in geography) IN GEOGRAPHY, at the University of New England, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 2).

RESEARCH ASSISTANT IN THE DEPARTMENT OF BOTANY, to work for one year on the acetate metabolism of the alga *Chlorella*—The Secretary, University College, Gower Street, London, W.C.1 (July 4).

LECTURER or ASSISTANT LECTURER IN INORGANIC CHEMISTRY in the Faculty of Science—The Registrar, The University, Manchester 13 (July 8).

BACTERIOLOGIST (honours graduate, majoring in bacteriology or biochemistry, with some years research experience in bacteriology) AT THE MEAT RESEARCH LABORATORY, Commonwealth Scientific and Industrial Research Organization, Brisbane, Queensland, Australia, to supervise the work of a small group investigating the metabolism of psychrophilic micro-organisms and the application of the results to the microbiology of chilled and frozen meat—The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 305/61 (July 9).

BIOCHEMIST, basic grade (with a first- or second-class degree in biochemistry, chemistry or physiology, or equivalent) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY, to carry out research on lipid metabolism in disease—The Secretary, The United Liverpool Hospitals, 80 Rodney Street, Liverpool 1 (July 9).

LECTURER IN ZOOLOGY in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (July 9).

LECTURER (with a good honours degree and preferably experience in the general field of applied mechanics) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, The University, Liverpool, quoting Ref. CV/T (July 9).

LECTURER IN THE COMPUTER UNIT—The Director, University of London Computer Unit, 44 Gordon Square, London, W.C.1 (July 10).

SCIENTIFIC OFFICER, Grade I (with an honours degree in mathematics or physics, and preferably relevant experience), AT THE WEAPONS RESEARCH ESTABLISHMENT, Salisbury, near Adelaide, South Australia, for research and development on frequency response techniques in optics—The Senior Representative (AV.63/1), Department of Supply, Australia House, Strand, London, W.C.2 (July 11).

SENIOR LECTURERS/LECTURERS (with an honours degree or equivalent, and in the case of Senior Lecturers a higher degree) IN APPLIED MATHEMATICS at the University of New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2 (London and Australia, July 15).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF BOTANY—The Registrar, The University, Edgbaston, Birmingham 15 (July 16).

SENIOR LECTURER IN PHYSIOLOGY; and a LECTURER (Grade I) IN BIOCHEMISTRY IN THE DEPARTMENT OF PHYSIOLOGY, University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (July 18).

CHAIRS OF BOTANY, CHEMISTRY, MATHEMATICS, PHYSICS and ZOOLOGY in the Royal Technical College of East Africa and proposed University College in Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 25).

LECTURER IN GENERAL PSYCHOLOGY—The Secretary of Faculties, University Registry, Oxford (July 25).

ASSOCIATE PROFESSOR (with a higher degree in pharmaceutics and wide teaching and research experience in physico-chemical or microbiological applications to pharmaceutics) OF PHARMACEUTICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, July 29).

ASSISTANT LECTURER (preferably candidate hoping to undertake research in the general field of genetics) IN THE DEPARTMENT OF ZOOLOGY—The Secretary, University College, Gower Street, London, W.C.1 (July 31).

LABORATORY SUPERINTENDENT IN THE DEPARTMENT OF PHYSICS, Fourah Bay College (University of Durham), Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (July 31).

LECTURER (with at least a good honours degree in geography and preferably experience of teaching geography in a university department) IN GEOGRAPHY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, July 31).

SENIOR LECTURER (with a Doctorate of Philosophy of Science conferred for research in some branch of physics) IN PHYSICS at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 15).

LECTURER IN MICROBIOLOGY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 31).

READER IN MATHEMATICAL STATISTICS at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 31).

ASSISTANT PROFESSORS (2) (with experience in the teaching of gross anatomy, and preferably willing to undertake some teaching in neuroanatomy, histology, or embryology) IN THE DEPARTMENT OF ANATOMY—Prof. R. L. de C. H. Saunders, Anatomy Department, Dalhousie University, Halifax, Nova Scotia, Canada.

BIOCHEMIST, basic grade—Dr. Walker, Biochemistry Department, Royal National Orthopædic Hospital, Stanmore, Middlesex.

BIOCHEMIST (with biological interests), to assist in research on the plasmin-anti-plasmin system—The Pathologist, Institute of Orthopædics, Royal National Orthopædic Hospital, Brockley Hill, Stanmore, Middlesex.

BIOLOGIST (with biochemical training), to assist in research on the plasmin-anti-plasmin system—The Pathologist, Institute of Orthopædics, Royal National Orthopædic Hospital, Brockley Hill, Stanmore Middlesex.

CHEMIST (with a good honours degree in chemistry or equivalent professional qualification and specialization or interest in inorganic analysis) IN THE GEOLOGICAL SURVEY DEPARTMENT, Tanganyika, for the analysis of rocks, minerals and ores, and the supervision of junior staff in routine analytical work—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.105/8/09/T.

EDITORIAL ASSISTANT (graded Assistant Experimental or Experimental Officer) (with G.C.E. "A" level in two science or a science and mathematics subject, or equivalent, if over 22 a pass degree, H.N.C., or equivalent, generally expected) IN THE PUBLICATIONS SECTION, Road Research Laboratory, D.S.I.R., Harmondsworth, Middlesex, for work involving editing and preparing for publication articles to technical journals, official booklets issued by the Laboratory, etc.—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting A.21/0A.

GRADUATE DEMONSTRATORS (male, with a first- or second-class honours degree or equivalent) IN CIVIL, MECHANICAL and ELECTRICAL ENGINEERING, PHYSICS, METALLURGY and BALLISTICS, at the Royal Military College of Science, Shrivenham, Berks—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. A.266/0A.

JUNIOR RESEARCH FELLOW IN PHYSICS—The Registrar, The University, Sheffield.

LECTURER IN PHYSICS, to teach Ordinary National Certificate and B.Sc. general degree students—The Principal, Peterborough Technical College, Eastfield Road, Peterborough.

LECTURER or ASSISTANT LECTURER, Grade B, IN MECHANICAL ENGINEERING—The Principal, Kingston Technical College, Fassett Road, Kingston-on-Thames.

RESEARCH ASSISTANT (preferably with experience in cytology), to take part in a programme designed to study human chromosome abnormalities and associated disorders—The Secretary, Genetics Department, The University, Sheffield 10.

RESEARCH BIOCHEMIST TO WORK IN THE DEPARTMENT OF HÆMATOLOGY on fractionation of blood components—The Secretary (R), The Hospital for Sick Children, Great Ormond Street, London, W.C.1.

RESEARCH FELLOW (preferably with an interest in solid state physics or nuclear physics) IN THEORETICAL PHYSICS—Prof. M. A. Preston, Department of Physics, McMaster University, Hamilton, Ontario, Canada.

RESEARCH STUDENT (physicist, electrical engineer, or physical chemist, with a first degree from the University of London) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, for work in the field of ultrasonics—The Registrar, Royal Technical College, Salford 5, Lancs.

SENIOR BACTERIOLOGIST (Principal Scientific Officer), and a BACTERIOLOGIST (Scientific or Senior Scientific Officer) with the East Africa Veterinary Research Organization, Kenya, for research into bacteriological and immunological problems relating to animal disease but primarily for basic studies on immunology with a view to developing a satisfactory vaccine for use in African conditions against bovine pleuro-pneumonia—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.195/214/02.

SENIOR LECTURER (with a first- or second-class honours degree or equivalent in physics, preferably with teaching and/or research experience) IN PHYSICS at the Royal Military College of Science, Shrivenham, Berks, to lecture to degree courses (and possibly specialized courses), and assist with laboratory classes—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting A.265/0A.

SENIOR LECTURERS, LECTURERS, or ASSISTANTS, Grade B (preferably with special interests in applied analysis, mathematical statistics, or other branches of applied mathematics) IN THE DEPARTMENT OF MATHEMATICS—The Academic Registrar, Hatfield Technical College, Hatfield, Herts.

SENIOR TECHNICIAN or TECHNICIAN FOR DUTY IN THE CLINICAL ISOTOPE LABORATORY—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

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